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ON CERTAIN ASPECTS OF HUMAN BIOLOGY

BY

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LECTURE I

ON THE GENETIC SIGNIFICANCE OF HEMILATERAL ASYMMETRY IN THE VERTEBRATE ORGANISM

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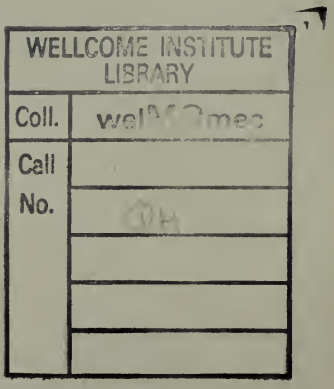

PREFACE

THE following Lecture was the first in a series of five Lectures "on Certain Aspects of Human Biology," delivered in April and May of this year, under the William Withering Trust.

Lectures IV and V "on Certain Aspects of the Physiology of the Red Blood Cell" have already been published.

Some of the problems dealt with in Lecture II "on the making of Use Acquirements" have been discussed in Chapters VIII and IX of my book, "Essays and addresses by a Surgeon" (Lewis & Co., London, 1930).

Lecture III "Genetics and the Preventive Medicine of the Future," will I hope be published later.

In now publishing Lecture I "on the Genetic Significance of Hemi-lateral Asymmetry in the Vertebrate Organism," I desire to express my appreciation of the honour conferred on me by the Medical Faculty of the University of Birmingham in appointing me William Withering Lecturer for 1932.

I also wish to record my admiration for the life work of William Withering, M.D., F.R.S.

I need not refer in detail to the many valuable contributions made by Dr. Withering to medical and scientific knowledge; to his introduction of Digitalis in the treatment of Cardiac Dropsy—to his botanical writings, to his friendships with Priestley and Erasmus Darwin, and other distinguished members of the Lunar Society of his day, to his labours as Physician to the Stafford Infirmary, and in 1799 to the General Hospital in Birmingham.

These and other details in Withering's life and work are referred to in the Life of Erasmus Darwin, also in the article by Professor Wynn in the Birmingham Medical Review of 1926, and more recently by Professor F. A. E. Crew, of Edinburgh, in his book "on Organic Inheritance in Man," which contains the addresses given in 1927 by Professor Crew as the first William Withering Lecturer.

LECTURE I

ON "THE GENETIC SIGNIFICANCE OF HEMILATERAL ASYMMETRY IN THE VERTEBRATE ORGANISM."

In this Lecture I propose to consider the problem of hemilateral asymmetry of structural characters as found in man and animals, and in the vertebrate organism generally. A short reference will also be made to asymmetry in invertebrates, in so far as this bears on the main problem.

I shall endeavour to approach the subject from the genetical aspect; that is I shall consider the nature of the factors which are concerned in asymmetrical development in the individual organism, and how they affect cell division in embryological development.

In an article in the Eugenics Rev. July 1929, Vol. xxl, No. 2, it was stated that "lopsidedness," i.e. hemilateral asymmetry, is the mark of a hybrid. Just as the hybrid through Mendelian segregation of heredity factors among its germ cells, produces offspring of different types, so also by an essentially similar process of qualitative cell division among somatic cells the body of the hybrid may itself be so built up as to show contrasting characters on each side of the mid line.

Here then we come across an important principle, namely the essential similarity between the segregative process as we see it at work in the phylogenetic and in the ontogenetic field.

In approaching the problem of asymmetry in the individual organism from the genetic point of view, we start from the fact that vertebrates, in common with certain invertebrate organisms, e.g. insects, are built up on a bilaterally symmetrical plan.

The two halves of the body, as far as the bony and muscular structures, the limb appendages, the sense organs and certain accessory structures like horns, teeth, and ears, are concerned resemble each other on each side of the median line. They are arranged in pairs on a bilateral plan.

In this Lecture we are concerned with certain exceptions to this rule of bilateral symmetry, and I propose to illustrate my remarks with examples of individual asymmetry drawn from horn curve in oxen, heterodactyly or odd-toedness in fowls, tail feather pattern, and colour feather pattern in pigeons and other birds, gyandromorphism in birds and insects, leading up to examples of asymmetrical eye colour, ear shape, and other characters in man.

But in addition to asymmetry between the two halves of the body of this bilateral kind, asymmetry may also occur in the " serial " or " end on " reproduction of vertebrate (and some invertebrate) structures as in the worm. I do not propose to deal in detail with this longitudinal or serial kind of asymmetry, except to mention one or two examples which bear upon the hemilateral aspect of the problem.

Before, however, we pass to a detailed consideration, a few words must be devoted to the embryological or developmental aspect of the subject.

The old Preformation theory of Roux and Weisman, (at any rate in its original form) has now been discarded by the modern school of experimental embryologists.

The process of growth and development, through the differentiation of parts in the fertilised egg cell, is now regarded as dependent on a number of factors, among which polarity, chemical differences in nutritive material, the point of entrance of the sperm cell, special capacities for regulating cell division and growth in certain localised areas of the blastoderm, are some of the more important.

The tendency to-day is in the direction of an epigenetic,

rather than a preformative theory of embryonic development. If we start then with the assumption that the development of a *recently* introduced character, such for instance as the extra toe, (when a four-toed breed of fowls is crossed with a five-toed breed), is controlled by the same heredity process as older *established* racial characters, e.g. colour pattern are controlled, during embryonic development,—then it is probable that an enquiry into these and other examples of hemilateral asymmetry, such as gynandromorphism in birds and insects, may throw additional light on the actual process and method of embryological growth.

For whatever be the nature of the process, (whether it be preformative or epigenetic) which starts the development of the fertilised egg cell on a bilateral plan of growth, the fact that an old established racial character is, for the most part, distributed *equally* to the two halves of the organism, while a newly introduced character, (such as an extra toe), is often distributed *unequally*, suggests the idea that the new factor, or gene, fuses with difficulty with the older established complex. In other words, that the old and new factors tend to segregate during the process of cell division which underlies the bilateral development of the embryo. The result being that asymmetrical distribution of the extra toe character occurs on the two sides of the body.

It looks indeed as if a condition of unstable equilibrum has been set up in the genetic machinery which controls embryological development, by the introduction of a foreign element or character into an old established racial genetic complex, and that this disturbance of equilibrum is in some way associated with the abnormal distribution of the character in question, to the two sides of the body.

It fact it is not altogether fanciful to regard the individual vertebrate organism as built up of two half individuals, mirror images of each other, fused along the longitudinal median plane, just as we regard a serially segmented organism, like a worm, as built up of a number

of potential worm individuals joined up in a linear or longitudinal series.

This " twin-like " view of the architecture of the vertebrate organism has also been recently suggested by Nathanial Hirsch ("Twins, Heredity and Environment". Thus on page 29 Hirsch speaks of Gynandromorphism, double monsters, hemi-atrophy, and the bilaterality of the normal individual, as instances of " mono-zygotic twinning."

This is important because it bears on the nature and meaning of the segregative process.

Thus in Mendelian segregation, a heterozygous germ cell containing alternative allelomorphic factors or genes derived from each parent, divides at a certain stage into two daughter gametes, one containing the paternal and one the maternal group of heredity factors, and these when fertilised give rise to two differently constituted individuals.

In the case of the irregular distribution of factors, or the lateral asymmetry which we are now considering, essentially the same process of segregation, or qualitative cell division, takes place, but it occurs at an early stage in blastomeric cleavage, when the two halves of the embryo are being laid down, with the result that one half differs from the other half in respect of the asymmetric character concerned.

In like manner during the process of somatic cell segregation in the apical cell of a plant shoot, this cell may undergo qualitative division, certain factors or genes may be eliminated or lost, and a shoot, different in genetic composition, the so called " Bud Sport " results.

The point I wish to emphasize is that, although the segregating process occurs among different kinds of cells, or units,—between two daughter gametes in the one case, and between the cells which form the two halves of the developing embryo in the other,—and although it takes place at different stages of development, yet the actual

process by which differentiation and asymmetry are brought about is essentially similar in both cases.

It is in this way that the study of hemilateral asymmetry in animals, and man, (and in some plants), becomes fundamentally an investigation into cases of unusual or abnormal segregation among the cell units, factors, genes, whatever their nature may be, which underlie and control the evolution of "characters" during individual or ontogenetic development.

Asymmetry of Horn Curve in Oxen.

We may now consider some examples which illustrate this hemilateral asymmetry, and we will commence with *Asymmetry of Horn Curve in Oxen.*

The old established *Longhorn* breed of cattle, now diminishing in numbers, is characterised by long tapering horns with a horizontal outward sweep, and a downward terminal curve. In a few exceptional individuals asymmetry of curve is seen on the two sides of the body.

In the *Hereford* breed moderately long horns with the same curve and sweep, with upturned tips in the bullock, or castrated male and the cow are also found, and the question arises as to the racial relationship between the Longhorn and the Hereford breeds. We also find the same tendency to a downward and an upward curve in individual cows from the *Red Lincoln* herd. *Welsh black cattle,* notably in Anglesea, also show the same horizontal outward curve with upturned tips.

Most writers on ancient breeds of cattle *agree that the large, long horned ox, Bos Urus or Primiginius, was succeeded in Western Europe and the British Isles by the smaller Celtic Ox, Bos Longifrons, with shorter horns, and that the now dwindling herds of wild white cattle, (at any rate the horned breeds) and the more recent Longhorns and Shorthorns, with their different horn curves,

* See Storr's "Wild White Cattle of Great Britain."

are derived from admixture between these more ancient breeds. It is in any case a suggestive fact that the most marked examples of asymmetry of horn curve in the same individual, which I have met, have occurred in the comparatively speaking, modern, Shorthorn breed of mixed ancestry.

The Eastern breeds of cattle, both in eastern Europe and parts of Asia and Africa to-day, have an upward type of horn curve. An examination of statues and frescoes representing the sacred Hathor Cow of ancient Egypt, and the more recently discovered archæological records in Chaldea, also show that the same upward type of horn curve, characteristic of Eastern breeds, existed at still earlier periods. Moreover the Bison of Eastern Europe and of the American plains is characterised by horns which, though short, have an almost vertically upward curve.*

Although the question requires further research, I think we are justified in putting forward the suggestion, as a provisional hypothesis, that the ancient races of cattle which originally inhabited Europe and Asia, from which our more modern breeds are derived by interbreeding, (with the exception of the hornless breeds—a mutational departure) were of two types as regards horn curve. One with an upward and one with a downward curve, with perhaps a third in which a wide spreading horizontal with a terminal forward and slightly upward curve was present.

Further that the frequent examples of asymmetry of horn curve in the same individual (when not due to previous injury), which occur in our cross bred breeds of to-day really represent a more or less successful segregational process occurring on the two sides of the same individual between this upward and downward type of horn curve.

* See Chapter on " Most Primitive Art " by Mr. C. Burkitt in Early Man.

With regard to the Bison, it is of interest to note that in some prehistoric drawings, notably in the Altamira Cave, and at Ariege in the Pyrenees the Bison is represented with horns of a horizontal forward curve with upturned tips, while in the Cave of La Greze in the Dordogne horns with a vertical upward curve are pourtrayed.

The curve of the fossilised horn cores in Pleistocene Bison Priscus (found in Kent) is horizontally outwards and upwards.

These facts suggest that in Prehistoric Ages races of Bison existed in Europe with horns of a different type of curve.

So far in these fossilised horn cores I have not come across any examples which suggest asymmetry of horn curve in the *same skull*.

Heterodactyly in Fowls.

Our next example of hemi-lateral asymmetry will be drawn from a study of "odd toes" or Heterodactyly in fowls.

In this case it is possible to control observation by experiment, that is by the results obtained through interbreeding.

A cross between a four-toed breed, such as the Game or Andalusian, with a five-toed breed like the Chinese Silky fowl, or the French Houdan, or the Dorking, produces F.1 chickens, among which the great majority possess five toes. Dominance of the extra toe, however, is not absolute, and a few asymmetric chickens appear with four toes on one leg, and five on the other.

Further it is an interesting fact that in these asymmetric individuals the fifth or extra toe appears in a greater proportion of cases on the left side.*

Barfurth's observations also confirm this fact, and more recently Punnett and Pease have shown that this tendency

*See papers, Journ. Genetics, Vol. X, No. 1, and Vol. XVI, No. 2, on "Polodactyly and Heterodactyly in Fowls."

to the left sided incidence of the extra digit may be characteristic of certain matings.

In crossings on a larger scale between G. Bankiva ♂, and the Chinese silky fowl ♀ all the F.1 chickens were five toed, that is the extra digit character was fully dominant, whereas in crosses between the less homozygous European breeds a number of the F.1 chickens were four-toed and some were asymmetric. But even in G. Bank, and silky cross, osymmetric individuals appear in the F.2 generation.

Selective Mating between Asymmetric Birds.

By selective breeding, that is by mating together asymmetric individuals from the Andalusian (four toed) with a Houdan (five toed) cross, through a series of generations, it has been possible to produce a considerable number of individual birds having more than the maximum number of five toes with which the experiment commenced. Six toes symmetrically disposed has occurred in a number of cases, and in others six on one side and five on the other.

A few birds have appeared with seven toes on both legs, and one or two cases with seven on one leg and six on the other.

It is of interest to note that when a number above the normal five, that is six or seven toes have been present on one side, the number on the other leg has been five or six respectively and does not drop to four.

For further details respecting these polydactylous and heterodactylous birds I must refer to my papers in the Journal of Genetics mentioned above.

I have already alluded to the occurrence of asymmetric individuals in the G. Bank Chinese Silky Cross. Speaking generally, although dominance of the extra digit is more complete in this than in the Andalusian-Houdan, or the Wyandotte-Dorking crosses, yet asymmetric birds appear in the F.2 and later generations in all the crosses. Super polydactylous birds, with six and seven toes symmetrically

placed, and a certain number of asymmetric birds also arise in all three crosses.

Extension to Other Toes.

The next point is one of considerable genetic interest.

As the selective breeding between these six toed and seven toed asymmetric birds goes on, the influence exerted by the extra or fifth digit factor, or Gene, on the heredity mechanism of the four toed breed, spreads to the other toes and does not remain confined to the extra digit. In later generations, not only do chickens appear with six or even seven digits symmetrically or asymmetrically disposed, but in some cases one or more of the three forward pointing toes may show stump like, clawless ends, or a claw, or claws, may appear on the side of these toes instead of at the end. Further these abnormal or clawless toes may themselves in some cases be asymmetrically placed. In other cases a forward pointing toe may be either abnormally long, or a mere stump in which some of the phalanges have been suppressed.

In one case the extra fifth digit was only connected to the side of the leg or metatarsus by a little tongue of skin.

The abnormal meristic division may extend to the leg, and an increased number of metatarsal bones may appear as in the Houdan-Andalusian hybrid pullet, described in the Journal of Genetics. (See above).

Extra Claws on the Wing Bones.

Further disturbance in genetic equilibrum may extend even to the fore limbs.

Although a horny claw is normally found at the extremity of the pollux in the wing of many individual fowls—an extra, single, or in some cases a bifid claw, has been seen on the first and second wing digits in some of these heterodactylous Houdan-Andalusian hybrids, recalling the free claws on the terminal digits on the wing of the primitive reptile-like bird, the Pterodactyle. In the

Pterodactyle however it is the little finger which forms the prehensile claw, and not the thumb.

Asymmetric Spur Development.

I will now refer to another example of hemilateral asymmetry, namely asymmetric spur development.

In the Journal of Genetics, Vol. iii, No. 3, I described the case of a pheasant with female plumage and no spur on the right side, and male plumage with a well developed spur on the left side. Microscopic examination of the sex organs showed an ovo-testis on the left side.

Attention was drawn to the failure of the ordinary theory of hormonic influence to explain the unilateral distribution of secondary sex characters in this case, and the suggestion was made that, in addition to the stimuli of the sex hormones, which are distributed equally to both sides of the body, there also existed in this gynandromorphic bird, a difference in susceptibility to hormonic influence in the tissues on the two sides of the body. Further, that this difference in susceptibility depends on genetic factors which segregate out during the early stages of blastomeric division, with the result that male *characters are developed on the one side and female on the other.

But this difference in receptivity to sex hormonic influence may show itself apart from any recognisable abnormality in the sex glands. Thus I have seen a number of cases of asymmetric spur development in hens, mostly on the left side, in which the normally placed left ovary, though somewhat atrophic, was otherwise normal, and no ovotestis or trace of any male gonad was present.

It is, of course, not very unusual for individual female fowls of several breeds, especially old hens, to develop spurs, (see Bateson—'Materials for the study of variation'), but in such cases a spur is present on both sides. It is the

* This bird was shown at the meeting of the British Association in Birmingham in 1913.

asymmetry of spur distribution, especially on the left side, together with the absence of any abnormality in the sex glands, which suggests a different susceptibility to hormonic influence on the part of the tissues on each side of the body.

In connection with these cases of lateral asymmetry in fowls, I may refer to the interesting hybrid, from a Light Sussex Rhode Island Cross, described by Professor Crew (Journal of Genetics, November 1928).

This bird was a male with marked skeletal differences, body colour, and general conformation on the two sides of the body. A small testis was present on each side, and there were no signs of ovarian tissue. Professor Crew thinks that this case was not one of gynandromorphism.

The point of interest to us is, that the marked hemilateral asymmetry in this bird was associated with cross breeding in the previous generation. The bird was a hybrid.

In connection with asymmetry of secondary sex characters, mention may be made of a case of congenital hypertrophy of the right breast in a boy aged 12, otherwise normal, recorded by Dr. A. Warner, Leicester, and in connection with skeletal characters a case of congenital hemi-hypertrophy of the right lower limb recorded by Dr. C. L. K. Herapath (Purves Stewart, " Diagnosis of Nervous Diseases," 7th Ed.)

Now if there be any truth in this idea of a difference in receptivity to hormonic stimuli in the tissues on the two sides of the body, then we might perhaps expect to find such a condition revealing itself in unequal response to other stimuli besides those supplied by the sex hormones.

It is true that the rashes and skin eruptions which result from the circulation in the blood stream of certain disease organisms and their toxins, e.g. the virus of the exanthematous fevers, are for the most part symmetrically distributed on both sides of the body.

Medical men have not, however, hitherto been on the look-out for asymmetrical distribution in these cases, and

it may be that examples of asymmetric distribution will be recorded when attention is directed to the subject of asymmetric tissue immunity.

I will recall, however, the case of the " Elephant Man," described by the late Sir F. Treves. It so happened that I had an opportunity of seeing and taking photos of this man at Leicester, at a very early stage of his deformity. At that period the effect of hyperpituitary secretion was much more marked on the right side of the body, head, and limbs, and, since it must have circulated equally on both sides, the marked difference in the response on the two sides must have been due to a difference in susceptibility on the part of the tissues on the two sides to the anterior lobe pituitary secretion.

In this connection Sir James Paget's paper on the relation between the symmetry and the diseases of the body, published in 1842 (see Med. Chir., Trans., Vol. xxv) is of interest, also the paper by Dr. William Budd in the same volume of the same Journal.

Asymmetry in Tail Feather Pattern in Pigeons.

I will now refer to certain examples of asymmetrical distribution of the tail feathers in pigeons.

In the three best known wild species—namely the Rock Pigeon (Col Livia), the Wood Pigeon (Col Columbas), and the Stock Dove (Col Oeneas), the twelve tail feathers, or rectrices, are always arranged symmetrically, six on each side of the mid line. The two central feathers arise at a slightly higher level, one on each side of the oil gland, on the dorsal surface of the tail.

In the Fantail breed, which has been established by selective mating with reference, among other characters, to tail development, a prize bird may show twenty-four, or more tail feathers, symmetrically or asymmetrically disposed on each side of the mid line.

The Homer, or Blue Rock, bred for flight capacity, has

a normal " fish " tail of twelve feathers symmetrically disposed.

The F.1 hybrids in a cross between the Fantail and the Homer, possess twelve tail feathers (the " Fish-tail " being dominant over the " Fan-tail ").

In the F.2 generation individual birds frequently show an asymmetric tail feather pattern, and even when apparently symmetrical, careful examination will often reveal that one or more of the feathers are double. The rachis divides into two, and two feathers arise from the same base or feather papilla.

Further, there is a tendency (as in the case of the extra toe in fowls), for these double feathers to appear more frequently on the left side. Thus out of a total of thirty-eight abnormal feathers, twenty-one were on the left side, and seventeen on the right.

Microscopic sections of the papillæ from which these double feathers grow, show that two papillæ may exist side by side in the same socket, surrounded by a common limiting membrane, at a very early stage of development.

Here then in double tail feathers and in asymmetry of of tail feather distribution in pigeons, as in hetero-dactyly in fowls, certain important facts emerge.

(1) The association between lateral asymmetry of characters and cross breeding and the hetero-zygous state, and

(2) The extension as inter-breeding progresses, of the influence of the gene or factor controlling the extra character (the toe or the tail feather) to other embryonic characters in the foot or tail, or even in the forelimb, as in the case of extra claws on the wings in fowls.

Thus it would seem that, in lateral asymmetry of bodily growth, a condition of unstable genetic equilibrium is brought about by the introduction of a new or foreign character (e.g. the extra toe or tail feather), which does not fuse easily with the old character or gene complex.

This instability settles down gradually, however, if no further dose of the new character is introduced, but if further inter-breeding between the heterozygotes occurs, then the cumulative influence of the factor for the new character spreads to the genetic or factorial composition of other characters, and irregularities in development begin to appear in, for instance, the forward toes in the case of the fowl's foot, and the individual feathers in the case of the pigeon's tail.

Asymmetrical Tail Colour Pattern.

In addition to irregularities in the number and distribution of the tail feathers, the colour pattern of the tail feathers may show hemi-lateral asymmetry.

Thus in a pullet, an F.1 cross between the old English Game ♂ and the Chinese Silky ♀, the feathers on one side of the tail were dark brown and those on the other white. This asymmetry of colour pattern may extend even to the whole or larger part of the body, thus a ♂ Homer pigeon, a cross between blue chequered and mealy chequered parents, showed a brown or mealy chequed pattern on one wing and a blue chequer on the other. (See Major Hurst's Collection).

In Professor M. Hachisuka's book "On Variations among Birds," kindly sent me by the author, a Japanese pheasant is represented with double spurs on both tarsi, and central tail feathers in which one side of the feather was black or melanistic, and the other normal.

Hachisuka also figures a red faced falcon (Melicrax Gaber) with, on one side melanistic, and on the other side normal plumage.

I have already mentioned the case of the gynandromorphic pheasant, with male plumage and a spur on one side and female plumage on the other. There is also the case of the bullfinch described by Hernrich Poll,* in which the same asymmetrical distribution of male and female

* Stygsber, Ges. Naturf. Freunde zu Berlin, 1909.

plumage was present. In this case the male plumage was on the right side, the female on the left, and Poll draws attention to the tendency in these hermaphrodite birds for the male characters to appear on the right, that is on the side on which the male gonad is generally situated. This is suggestive when we remember that normally in birds the rudimentary ovary atrophies on the right side, and the left ovary alone remains functionally active in later life. Prof. Hare of the Royal Veterinary College, London, informs me of a Budgerigar described by Mr. Norman in "Cage Birds," March 1932, in which the body was Cobalt blue on the left side and green on the right. Mr. D. C. Seth Smith has also recorded other examples. Herr C. Balser, Langen, Darmstadt, has also bred two such Asymmetric birds, and in one case gives the pedigree which shows a Cobalt father mated to a Green mother.

Asymmetry of Coat Colour Pattern in Dogs, and Guinea Pigs.

I have come across a number of cases in terriers, especially the fox terrier breed, with a white ground colour and black, or black and tan markings, in which the distribution of the coloured markings was limited to one side of the face, ear and head, and was sharply defined by the mid line. An enquiry into the ancestry of such individuals generally reveals a history of previous cross breeding, and the asymmetrical distribution seems to be especially frequent in the Sealyham breed.

Although this sharp midline limitation of colour pattern occurs on the face and head, I have never observed it on the tail. This is an interesting point, which will be referred to later.

Kastle and Buckner (American Naturalist, Vol. xlvi, September 1912) describe the case of asymmetric coat colour pattern in a female guinea pig, in which the colour

pattern resembled, but was on the opposite side of the body to that of its asymmetrically marked mother.

Gynandromorphism and Hemilateral Asymmetry in Insects.

A considerable number of cases of hemilateral distribution of primary and secondary sex characters have been recorded in gynandromorphic insects. Examples of ants, butterflies, and moths showing more or less complete male characters on one side, and female on the other, can be seen in Lord Rothschild's Tring Museum and in other museums. These are of special interest because, as far as is known, sex hormones derived from the male and female sex gonads pay a very insignificant part in embryological, i.e. somatic development in insects. The distribution and development of the sex characters, primary and secondary, seems to be controlled by the sex chromosomes in these invertebrates. Silkworm larvæ showing the same kind of asymmetry have been described by Japanese observers. The work of Goldsmicht and others have thrown much light on the genetic nature of these intersexual individuals.

Segregation among gametes, under the control of the sex chromosomes which normally leads to wholly male and wholly female individuals—in these abnormal intersex insects apparently produces two half individuals each of a different sex, united more or less completely into one individual organism.

In " The Science of Life " (Wells, Huxley and Wells), Chap. VI, Book 4, p. 371, a diagramatic scheme is figured of the sex chromosome mechanism by which these sex-mosaics are produced.

A curious case is also mentioned in which a high proportion of Gynander individuals arises among Chrysalids of swallow tailed butterflies, which have been shaken while in the chrysalis stage. In Gynanders so produced, the sex characters are patchy, and are not apparently due to the same irregular disposition of the sex chromosome,

as in ordinary Gynander insects. The unequal distribution of the factor or influence which controls the development of the secondary sex characters to the two halves of the organism is, in this case, apparently associated with the mechanical shock previously received during the chrysalis stage.

The Higher Incidence of the Extra Toe and other Asymmetric Characters on the Left Side of the Body.

I have already mentioned that, in asymmetric birds, there is a marked tendency for the extra digit or digits to appear on the left side of the body, although, as Punnett and Pease have shown, this tendency is associated with certain matings and certain strains.

The same seems to be true of heterodactyly in man, see Svoerdrup, Journal of Genetics, Vol. xii, 3, 1922. It is of interest to find that Dr. G. C. Little, in a paper on "The Effects of Selection on Eye and Foot Abnormalities occurring among the Descendants of X-Rayed Mice," American Naturalist 1931, lxv, 370-375, describes a considerably higher percentage of abnormality in the left anterior and left posterior, than in the right anterior and right posterior limbs amounting to 58·9%. Murray and Bagg have also reported on the same lines.

These observers also draw attention to the association between eye and limb, and other abnormalities, in different individuals belonging to radiated families.

These cases are of special interest because the abnormality is presumed to have been started by radiation of the bodies of animals belonging to preceding generations. The defects, in fact, have been experimentally produced. What is the meaning of this greater incidence on the left side?

I have already mentioned the atrophy of the right and the development of the ovary to maturity on the left side

in birds, and the greater incidence of male characters on the right side in hermaphrodite birds. In man the highly developed brain is an asymmetrical organ. The centres for written and spoken language are normally situated in the left cerebral hemisphere. It would seem then that asymmetry is associated with increasing complexity of evolutionary development. Further it would seem that the condition of unstable genetic equilibrium associated with cross breeding affects the left side of the organism more than the right, and judging from Little's results, the same seems to be true of experimentally produced characters. We are not at present able to say why this should be so, just as we cannot say why the spiral hair twist in the tail of the ox is always anti-clockwise, or why it is the left tooth in the Narwal which usually undergoes excessive development, while the right remains concealed in the jaw. In the ♀ Narwal both remain concealed. The over development in the male, of the unilateral tooth with its spiral L. to R. curve represents in fact a secondary sex character.

Other examples can be mentioned; thus an enquiry into the number and position of supernumerary teats in Guinea Pigs gives a higher incidence on the left side.

On the other hand some asymmetric abnormalities appear in greater frequency on the right side. As we shall see later this is the case with Darwin's Ear Tubercle among the Swedes and Finns, where it is twice as frequent on the right side.

Hemilateral Asymmetry and the Presence and Absence Hypothesis.

Nor is it necessary in the case of the hemilateral asymmetry of any character, such as the fifth or extra digit in asymmetric fowls, to suppose that the irregular distribution of the extra digit must depend on the complete presence or absence of the heredity factor on the corresponding side only. It may be that the factor, or gene

complex, is present on both sides of the developing embryo, but in greater dose or volume, or in a more active state on one side than the other, thus accounting for the asymmetrical development.

Thus if we suppose with R. A. Fisher (Biological Rev. Vol. vi, No. 4) that the difference between a Dominant and a Recessive character in any Heterozygote depends on the action of two different chemically active substances, then much may depend on the activity and diffusibility of these substances, and on the way in which they influence cell division, i.e. slowly, and at short range, or more quickly and with a longer range action.

Asymmetry in Man.

Ear Pattern.

We must now consider some examples of hemi-lateral asymmetry in man.

We will commence with asymmetrical ear pattern.

On January 20th, 1928, a leading article appeared in the "Times," on a "New Use for Ears," pointing out that French criminologists were making increasing use of ear pattern as an identification mark for criminals. In a subsequent letter to the "Times" (January 30th) I endeavoured to point out the anthropological and genetic value of such records.

If we believe that hemi-lateral asymmetry of ear pattern in man, like asymmetry of toe pattern in fowls, and asymmetry of tail feather pattern in pigeons, is in some way associated with cross breeding, and the heterozygous state, then a careful study of ear pattern, and other examples of asymmetric distribution in the human species, is likely to lead to important results.

Without going into the embryological and morphological facts regarding the growth and development of the auricle during fœtal life in man, it is enough to state that the abnormalities with which we are now concerned

in this study of asymmetry, chiefly affect the lobe, and the contour of the helix.

Individuals vary greatly in size, shape, and attachment of the ear lobe, so much so, that we might divide the population roughly into three categories—" lobed " and lobeless," with an intermediate " semi-lobed " group.

It is true that the lobe is rarely absent, but it varies from a well developed appendage free of the cheek, to a mere skin vestige which continues the lower cartilaginous edge of the concha on to the side of the face, while in some individuals and in some races it may be entirely absent. In some families the familial incidence of the lobe pattern derived from the parents may show segregation in the children of the F.1 or the F.2 generation.

Thus in one family (a), in the father, the ears are large and long, vertically placed with a well developed lobe, and apparently symmetrical, though careful measurements show that the right ear is slightly longer from above downwards than the left. In the mother the ears are shorter in the vertical measurement, and the lobes are only slightly developed. From this mating a son resembles the mother, the ear lobes are only moderately developed, with a slight asymmetry on the two sides. A daughter resembles the father, the lobes are well developed and symmetrical.

In a second family (b), a curious asymmetric abnormality of the helix was observed. An overhanging incurved cartilaginous lip was present in the left ear only, in two daughters in a family of eight children, the offspring of a father and mother both with moderately developed ear lobes.* This asymmetry of ear pattern was also associated, in this family, with segregation of eye colour, skin, and hair colour. In the other brothers and sisters, some had dark eyes and black hair, and one child was fair with blue eyes and red hair.

* The condition of the helix resembled the helix of the Ear of Bushmen and Hottentots as described by E. A. Hooton. (Up from the Ape. Allen & Unwin, p. 434.)

Other examples could be mentioned in which asymmetry of ear pattern is associated with interbreeding between parents belonging to the "lobed" and "lobeless" types of ear pattern.

Abnormalities of ear shape of an asymmetrical kind may show a familial incidence: thus Ruggles Gates ("Heredity in Man") gives a chart showing the inheritance through four generations of a bilobed condition affecting the right ear only.

Another family has been recorded by Kindred, in which a pit in the skin of the helix in the left ear only, was transmitted through four generations.

Ruggles Gates also mentions Hilden's study of Darwin's Tubercle in the population of Finland and in Swedish School Children.

Hilden found that when asymmetrical, the tubercle was twice as frequent in the right ear as in the left, in these peoples.

Asymmetry of Ear Shape in Mentally Defective Individuals.

Although accurate records are somewhat difficult to obtain, there seems to be a general opinion among experts, that lateral asymmetry of ear shape is, among other characters, more common among imbeciles, idiots and persons suffering from various degrees of mental deficiency, than in the normal population. Thus Dr. Tredgold, in his book on Mental Deficiency, figures cases; and I noticed several among the photographs in Dr. T. Brushfield's collection of records from the Fountain Hospital, now at the Royal College of Surgeons.

Dr. E. B. Strauss of the Marburg University Psychiatric Clinic (see Journal of Mental Science, October 1930) reports that among 110 male cases of hereditary defect:

In 76 ear lobes were adherent
12 the ears were asymmetrical
22 ears very small
13 ears of a simple pattern
10 Darwin's Tubercle present.

In a series of bilateral Photographs of the Ears of a number of inmates of the Leicestershire Mental Hospital, kindly obtained for me by Dr. Drury, asymmetry in the same individual occurs in seven cases.

When considering asymmetry of ear shape in the individual, some reference must be made to the wider or racial aspect of the problem.

The Yellow (Mongolian) races of mankind are characterised by "lobeless" ears; see F. G. Crookshank, "The Mongol in our Midst." While in the Black (Negro) races, the ears—though small (Ruggles Gates)—have a moderately developed lobe and are longer in the vertical measurement.

The White (Caucasian) races seem to be intermediate as regards ear shape, and vary considerably among different peoples and among individuals belonging to the same group, both as regards ear pattern, and ear lobe development.

Crookshank, and some other writers, go further and relate ear shape and other characteristics, such as cranial index, posture and palmar lines, to a kinship with one of the three main anthropoid ape groups, the Orang, the Chimpanzee, or the Gorilla. A study of the ear pattern among these three species of apes certainly indicates that the ear shape of the Gorilla is not only characteristic, but that it varies considerably from that of the Chimpanzee and the Orang, which are both more or less lobeless. While perhaps an evolutionary association may be more easily established in the case of the Mongolian races and the Orang, and perhaps between the Gorilla and the Negro races, than between the white Caucasian race and the Chimpanzee, there is the fact that examples of Mongolian

idiotcy are found among the Whites and Mongols, and are not recorded among the Negro or Blacks, or have only been recorded in a few doubtful cases. The further fact that variety of ear pattern seems to be more common among white peoples than Blacks and Mongols would be in harmony with a more mixed ancestry in the Whites, and if it be true, as we suggest, that hemilateral asymmetry of any character is associated with the heterozygous condition as regards the same character, then asymmetry of ear pattern in the same individual should be more frequent among the Whites than among the Black or Yellow races. The study of asymmetry of ear shape, like that of asymmetrical eye colour, has the great advantage that it is largely independent of environmental influences.

Further studies into the Racial Significance of Ear Pattern are very desirable. If the association between lateral asymmetry and cross breeding which is now put forward is sound, then the primitive pure bred races of mankind should show less Asymmetry in Individual Ear Pattern than the much mixed Caucasian or White races. I also think that a study of Ear Pattern which is distinctive in the three Anthropoid groups, the Orang, the Chimpanzee, and the Gorilla, would also show an absence of Asymmetry in individual members of these three groups.

Asymmetry of Eye Colour (Heterochromia Iridis) in Man and Animals.

Twenty years ago (Journal of Genetics, June 1912) I gave examples, with pedigree charts, of a number of families in which, from matings between blue eyed (simplex) and brown eyed (duplex) parents, one or more of the children in a family showed asymmetrically coloured eyes, one blue and one brown in the same individual. In some cases a sector, or ray of brown coloured iris appeared in a blue eye, with the other eye wholly blue.

Here then we have to do with an asymmetrical distribution of the gene complex, or factor, which controls pigmentation on the anterior surface of the iris on the two sides of the growing organism before birth.

Further, among the many characters which emerge during the growth of the individual, that of eye colour is probably but little influenced by environmental factors such as nutrition, hormones, or use, hence we are probably right in supposing that asymmetric pigmentation here depends on the unequal distribution of genes or factors during the very early stages of cell division, those which underlie the bilateral development of the embryo.

Other abnormal conditions affecting the eyeball of an hereditary kind may also show asymmetry, e.g. Coloboma Iridis and Microphthalmos.

Professor Seligman (British Association Meeting, London 1931) in a paper on Human Hybrids, mentions that the epicanthal fold in certain F.1 individuals from a cross between Chinese and White may be present on one side of the face only.

*Mr. Bickerton has collected a large number of familial records showing the widespread influence of Heredity in Ocular defects.

Miss Fleming informs me that among the children born of European (White) mothers and Chinese (Mongolian) fathers in Liverpool, she has come across one little girl in whom a dark brown iris with Mongoloid orbital features were present on one side of the face, and a blue eye and European features on the other.

The subject is not without a certain historical interest—thus Victor Hugo in one of his romances describes the heroine as having one blue and one brown eye, and he justifies this by citing like cases among historical characters, among others Mary, Queen of Scots, who he says had eyes of a different colour.* This if true, may in

* Miles Bickerton. Address to the Eugenics Soc., March 1932).
* Letter by Rev. V. A. Boyle, " Times," October 27th, 1930, p. 8.

part account for the discrepancy in the descriptions by various writers as to the colour of the Queen's eyes.

The fact that asymmetry of eye colour occurs with much greater frequency among the individuals of certain families, in which cross breeding has taken place between Simplex and Duplex (Hurst) parents, than in the general population confirms the suggestion that the irregular distribution of Iris pigment on the two sides of the body is associated with some general disturbance of the genetic balance between these two allelomorphic characters.

The absence of environmental influence in the case of Eye Colour is in harmony with this view.

Asymmetry of Eye Colour in Animals.

But asymmetry of eye colour in the same individual is also found in animals.

The so-called "wall-eye," with complete or partial absence of anterior iris pigment occurs in certain horses, especially those belonging to the patchwork, multi-coloured breed found in circuses. In dogs it is characteristic of the old English sheep dog, and the Collie breeds. It occurs also in Great Danes and Dalmatians. I have also noticed it in a few cats.

In the Journal of Genetics, June 1912, I recorded the case of 100 wild rabbits shot on a small uninhabited Orkney Island (Holme of Scotness). Of this number four individuals showed asymmetric pigmentation (or partial "wall eye") in one or both eyes. Eighty-one were of the wild brown-grey colour, and nineteen showed some white patches or irregularity of coat colour, and three were of the Dutch pattern. Further, of the four "wall eyed" individuals three gave the Dutch pattern of coat colour.

It is known that "wall eye" tends to occur in the Dutch breed, and it is also known that some white, or brown and white, ? Dutch rabbits were turned loose by a farmer on this island six years previously. Thus in this case the

evidence strongly points to an association between asymmetric eye colour and cross breeding.

Lop and Half-Lop Ear in Rabbits.

Darwin (Animals and Plants under Domestication, Vol. I, p. 131), remarks on the asymmetry of the half-lop ear in Rabbits and the irregularity of its inheritance. In this case there is much evidence as to the origin of the ear condition in cross breeding between the various domestic breeds, which have all been derived by artificial selection from the wild rabbit with small erect ears. Darwin also mentions the fact that " half-lop " is associated with a difference in the direction of the auditory meatus in the skull. He also quotes Anderson as describing a breed of sheep with only one ear. A condition of " Prick and Drop " Ear is sometimes seen in individual dogs of the Terrier breeds.

The Meaning of Hemilateral Asymmetry.

The cases of hemilateral asymmetry which have now been mentioned; for instance horn curve in oxen, polydactyly, and heterodactyly in birds and man, asymmetry of ear shape and eye colour, irregular distribution of coat colour in animals,—all these are associated with qualitative cell division at a very early stage of embryonic development. They do not depend on nutritional and circulatory, or on hormonic factors. This seems to be true also of gynandromorphism in insects. Gynandromorphism in birds, on the other hand, and the asymmetric distribution of male and female secondary sex characters, as in the case of my pheasant and Poll's bullfinch, seem to depend partly on chromosomal or genetic and partly on hormonic influences.

But this only carries the problem one step further back. The cause of the abnormal arrangement of the sex gonads in such cases still awaits solution.

The failure of the right ovary to reach full development in birds previously mentioned is of interest. It cannot be due to hormonic influence, and must in some way depend on chromosomal factors. Perhaps an asymmetric character, which probably originated in a mutational departure from the normal, has now in birds become a fixed and permanent racial feature.

The available evidence suggests that the somatic tissue cells on the two sides of the body in these exceptional cases differ in their susceptibility to, or capacity of response to, hormonic influences, especially to the stimuli derived from the male and female sex gonads. The tissue cells differ in fact in innate " maleness and femaleness," and this difference in secondary as in primary sex characters must depend on factorial composition.

The experiments of Roux and other observers on the Eggs of Frogs have shown that the two half individuals which, in the normal embryo are united along the median line, represent at an earlier stage two potentially whole individuals, since each of the first pair of blastomeres, if shaken apart, can develop into a whole organism. In certain cases, however, in which blastomeric division is disturbed, these two potential wholes may develop unequally. The question is, what is the nature of the disturbing element which causes this unequal cell division, and later the hemilateral asymmetry.

It looks as if the segregative process which is responsible for the unequal distribution of parental characters to different daughter gametes, and which normally ceases with maturation of the gametes, is carried on into zygotic development in these abnormal individuals where it results in mono-oval twins, or double monsters, or hemilateral asymmetry, according to the degree of the segregation.

In connection with this view of the organism as built up of two half, but potentially whole individuals united along the mid-line, it is of interest to find that Huxley (Essays in Pop. Science, p. 240, 1927) states that a large

proportion of individuals with transposed viscera are members of pairs of identical twins.

I have already said that when lateral Asymmetry occurs in zygotic development, the evidence suggests that a state of genetic instability has been set up by the introduction of some foreign factor into an old established genetic mechanism, through cross breeding.

This suggestion receives some confirmation from the fact that dominance is frequently incomplete in the F.1 generation in such cases. Thus in the Houdan-Andalusian and in the English Game Silky Crosses in fowls, although the majority of the F.1 chickens were five toed a small minority were four toed, with a few asymmetric birds. In the F.2 and later generations the number of heterodactylous birds increased.

In the Gallus Bankiva Silky Fowl Cross, dominance in the F.1 generation is more complete, and the number of asymmetric birds fewer in the F.2 and later generations.

The Brain and Hemilateral Asymmetry.

A word must be added about the brain. The vertebrate brain is a dual organ, built up of two half brains. The human brain exhibits asymmetry of structure and function in a higher degree than the brain of the lower animals, and even that of the anthropoid apes.

As already stated the centres for written and spoken language are normally located, in man, in the left cerebral hemisphere, which—owing to the development of cross communicating fibres in the course of Phylogenetic evolution—now controls the movements of the right arm and hand, and this in man is the dominant or leading forelimb.

Some important psychological problems may be bound up with this question of structural and functional asymmetry in the human brain, such for instance as those of dual and multiple personality, left handedness, and stammering, etc. It is also an interesting fact that

asymmetry should be characteristic of the anterior, the advancing and exploring end, rather than of the posterior end of the organism.

Thus, although marked hemilateral asymmetry of face colour, sharply limited by the mid-line,is fairly frequent in the Terrier breed of dogs, I have never seen sharply limited hemilateral distribution of coat colour on the tails of dogs.

CONCLUSION.

From this study of hemilateral asymmetry in the vertebrate organism we arrive at the conclusion that the segregative process, though it occurs among different units, cells, or cell groups, is yet essentially the same process in each case.

In Mendelian segregation the process takes place during phylogenetic development among reproductive germ cells or gametes, and results normally in the production, from the same mating, of individuals possessing different genetic constitutions.

If the segregative process takes place among the somatic cells of the developing embryo at the stage when the bilateral plan of growth is laid down, then it gives rise to a single individual made up of two half individuals differing from each other, and hemi-lateral asymmetry results.

In an address to the Oxford Medical Society, (see B.M.J., February 24th, 1912), I drew attention to the fact that variation, that is the assumption of new characters by any organism, cell group, or cell, is the result of a process of segregation occurring among units of a certain order, size, or complexity.

In hemilateral asymmetry those units are the body cells which form the two halves of the individual vertebrate organism, and the differentiating process takes place at that stage of embryonic growth when the bilateral scheme of development is laid down.

Modern research has shown that, in the development of the fertilised egg cell, epigenetic factors supplement performative influences at a certain stage of embryonic growth, while performative processes again come into play when the germ cells are in their turn formed within the body of the zygote. Segregation plays an important part at both ends, and in both processes.

Eggs of the " regulation " type and the " mosaic " type vary in the stage at which the epigenetic process comes into play, and it may be that in these asymmetric embryos a condition of unstable equilibrium is present, due to failure of allelomorphic fusion, in which qualitative cell division continues to operate up to a later stage of development than is the case in normally developing homozygous egg cells.

Hemilateral asymmetry in fact seems to be associated with the introduction of a foreign factor or gene complex through cross breeding, that is with the heterozygous condition.

It may be that the interbreeding between races and peoples of different genetic constitution, which is now taking place in different parts of the world, will bring about a further degree of asymmetry in the human beings of future generations, not only in external form and features, but in the unifying organ, the human brain.

The extent, however, to which such asymmetry can develop will be limited by the necessity for integration and a unified personality.

It is in this direction that the study of asymmetry in the individual becomes of special interest to students of genetic and racial problems.

We have shown, for instance, that the gene complex or factor, which controls the development of the extra digit in fowls, multiple tail feathers in pigeons, odd eye colour in man, is not only hereditarily transmitted as such, but that under certain conditions, e.g. in selective breeding, its

influence tends to spread to other factors and to other structures and characters.

This fact indicates the extreme complexity of the heredity process, and the mutual interdependence which exists between the factors concerned in it.

Whatever may, in the past, have been the directional influence, within or without the organism, which determined the bi-lateral plan of development in the vertebrate organism, that plan or scheme of growth has at any rate provided a field for the occurrence of bilateral asymmetry and greater facilities for the making of better spatial adjustments. For this reason also the study of hemilateral asymmetry deserves careful attention.

I have alluded to the high incidence of asymmetry in individuals who show signs of degeneracy in the medical sense. Thus asymmetry of ear pattern seems to be more than normally frequent in imbeciles, mongols, and other types of mental and physical abnormality.

This is what we might expect, if degeneracy and other defects of an hereditary kind arise out of, or are associated with, that state of genetic instability which is linked up with incompatability of allelomorphic characters in varying degree.

When describing asymmetry of coat pattern in dogs, I alluded to the fact that the asymmetric distribution was more commonly seen on the head and face than on the tail. This, in genetic terms must mean, that the factors, or gene complexes, which control the distribution and development of structural characters at the anterior, or head end of the organism, are more disposed to segregate out irregularly, i.e. to irregular distribution, than those which control the development of the same characters at the posterior or tail end.

This may perhaps be due to their greater number and complexity in the former case.

The human brain, for instance, is a very asymmetric organ.

Although our present knowledge of the way in which genes, and gene complexes, control cell development is incomplete, the evidence, such as it is, points to a capacity for initiating or activating cell metabolism and cell development in various directions, perhaps in somewhat the same way that chemical activities are started by enzymes.

We may also ask whether this attempt to explain the occurrence of hemi-lateral asymmetry as due to some interferance with the normal heredity process, in which genes or factors are distributed equally to the two halves of the growing embryo, fits in with, or runs counter to, the so-called " organismal " theory of development.

The root idea underlying the " organismal " theory is that the organism functions as a whole, and that any attempt to explain ontological development by a " particulate " or " cellular " theory is likely to fail.*

It is no doubt true, that, in a certain sense, and up to a certain point, the organism does function as one whole.

That is to say, among the different orders of units, of different size and complexity, with which evolution is concerned, the unit or organism of larger size and greater complexity, embraces and controls the activities of its component smaller units, according to the degree of integration possessed by the larger combination.

At the same time it is also true that the smaller component units also retain an autonomy of their own. The somatic cells which compose the organism make their own " use-acquirements " which in many cases, though not in the case of the nerve cell, are transmitted to cell descendants, though not, it would seem, in the human subject to the germ cells, and so to offspring.

* See E. S. Russell. The Interpretation of Development and Heredity.

Hemi-lateral asymmetry of any given character constitutes an exception to the normal working of the epigenetic process by which the embryo develops. The evidence suggests that the asymmetry depends on some irregular or unusual qualitative cell division at an early stage of blastomeric growth, whereby the factors, or genes responsible for the development of the character in question are distributed *unequally* to the two sides of the organism.

The fact that this irregular distribution is apt to occur when normal genetic stability in any heterozygote has been upset through the introduction of a new character, suggests that we must seek for its explanation partly at any rate along cellular or particulate lines, for in this case the controlling influence of the organism as a whole seems to be deficient.

The question also has been asked, what is the relationship, if any, between this unequal distribution of a given character to one side only of the body, and those defects, such as cleft palate, meningocele, and many others, which affect the median plane of the organism and which, like hemilateral asymmetry, are also hereditarily transmitted?

If we look for some antecedent genetic factor in both cases, then it would seem that the departure from normality in the first is due to some interference with the developmental mechanism by which the gene or gene complex, which controls the development of the character in question, is distributed unequally to the two halves of the growing embryo, while in the second case there is some defect or arrest of development in the gene or genes which control the process by which fusion of, for instance, the two sides of the palatal shelf is brought about in the mid-line.

In the one case there is irregular distribution of normal genes, in the other, some defect or arrested development in the genes themselves.

But whatever may be the nature of the method by which the genetic mechanism works, I am hopeful that a study of exceptional cases—as for instance hemi-lateral asymmetry—may throw further light on the problem of hereditary transmission.

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ON CERTAIN ASPECTS OF HUMAN BIOLOGY

BY

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LECTURE II

ON THE MAKING OF USE ACQUIREMENTS; THE NEURO-PSYCHIC & OTHER RESPONSES

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PREFACE

THE following Lecture II, "On the Making of Use-Acquirements" is mainly concerned with a consideration of the way in which the changes in cell structure and function which constitute Use-Acquirements, are brought about.

The suggestion is made that such acquirements are the outcome of a process of variation and selection occurring among *Intra* cellular units.

These acquired characters are considered from the point of view of the individual organism and the cell or cells which undergo the change.

The response to environmental stimuli made by the Nerve cell, the Muscle cell, the Epithelial cell, the Cancer cell, the Bone cell, and by certain unicellular organisms, together with the acquired Immunity Reaction, are discussed from the same point of view.

The question of the transmission of Use-Acquirements, made by somatic cells, to the descendants of these body cells on the one hand, and to the Germ cells (and so to offspring) on the other, is discussed, and the suggestion is made that the apparent discrepancy in the evidence presented on the two sides, may be due to innate differencies in the susceptibility of the Germ cells to environmental influences in organisms of different types.

Certain possible objections to the theory of the making of Use-Acquirements here proposed are dealt with, and in conclusion it is claimed that, although this study of acquired characters may not throw any new light on the origin of the variations or mutations on which evolution is based, it does suggest that Ontogenetic development and Phylogenetic evolution are essentially similar processes, though concerned with units of a different order.

The factors concerned, namely (1) Cell or individual potentiality. (2) Environmental influence, and (3) the reaction between them, are alike in both cases.

LECTURE II

ON THE MAKING OF USE-ACQUIREMENTS. THE NEURO-PSYCHIC AND OTHER RESPONSES.

In this Lecture I shall discuss the different ways in which use-acquirements are made by the individual cells and the cell groups which make up the individual organism.

The suggestion I shall put forward may seem at first sight, somewhat strange, but I must ask my readers to withhold their verdict until the evidence has been presented.

There will, however, be general agreement that the method by which the individual organism learns, or in other words, profits by experience, constitutes one of the most interesting aspects of the evolutionary and developmental process.

Much of our philosophy of life depends on the nature of our conception of what constitutes a use-acquirement, and before proceeding further, it will be necessary to explain what, for the purpose of our present enquiry, we mean by that term.

Ever since Weisman's day there has been a tendency on the part of many biologists to regard the differences which arise *among* organisms, and those which arise *in* organisms, as the outcome of an essentially dissimilar process. In other words, we are apt to draw a fundamental distinction between co-called innate and acquired characters, as far at any rate as their mode or origin is concerned. In this Lecture I shall hope to indicate reasons for thinking, that the method by which the individual cell, or the groups of cells, which make up the individual organism, become adapted to environmental change is essentially the same kind of process as that by which organisms and species become adapted to changing conditions of life during racial evolution.

Both are the outcome of an adaptive response, but that response may be described as "*Direct*" in the first case "*Indirect*" in the second.

The difference between the "Direct" and the "Indirect" form of response depends however, as we shall see later, on the kind or order of the units concerned in the reaction and on the conditions under which the response is made. In "*Indirect*" adaptation, brought about by the struggle for existence and by natural selection, the units concerned are, for the most part, individual organisms, or groups of organisms, while in "Direct" adaptation, through use-acquirement, they are the physiologically differentiated parts of which individual cells are composed. The question we have to answer then is: Does the process of selection which promotes adaptation by ensuring the survival of the more fit varieties among individual organisms, also control adaptive change in the living elements which compose the individual cell?

Now we know, more or less, what the conditions are under which selection takes place among organisms when they are exposed to a changing environment, to which they are undergoing adaptation.

These conditions are:—

1. Struggle for existence.
2. More or less rapid production of organisms.
3. Destruction of the less well adapted organisms, through the operation of selection either natural or artificial.

We shall find the same conditions present during the process by which the individual cell undergoes adaptation to environmental change, or in other words makes "use-acquirements."

Thus "Use-acquirements" are made during the functionally active phases in the life of a cell, in which wear and tear and reconstruction of cell parts, especially in the cyptoplasm is going on. This provides not only the

opportunity for the origination of new, and for the re-arrangement of old parts, but also for the selection of those elements which are more fitted to survive under the new conditions. In both cases, in "*Direct*" as in "*Indirect*" adaptation, it is the environment which acts as the selecting agent.

It will be noticed that in both cases the process rests fundamentally on reproduction or multiplication of units or parts in association with qualitative differences or variation among the units which are multiplying.

In racial evolution this multiplication takes place among germ cells, and the opportunity is thus given for the appearance of differences between these reproductive cells of a qualitative kind.

In use-acquirement the opportunity for the origination of qualitative differences is provided by the multiplication of the living molecular groups or structural units (the biophores in Adami's language) which compose the individual cell.

There is, however, one important difference between the "*Direct*" and the "*Indirect*" process—it is this:—In the former the testing and selecting process takes place during the life of the individual cell, in the latter it occurs during the lives of the cell descendants of the cells which vary, *i.e.*, among the individual organisms which spring from the varying germ cells, and which function as the hosts of the germ cells of the next generaton.

It is for this reason that I have called the first "*Direct*" adaptation and the second "*Indirect.*"

The two processes resemble each other, however, in their mutual dependence on the same two factors, cell potentiality, and environmental influence, and on the reaction between them.

But there are also further points of resemblance. Before the germ cell undergoes mitotic division, a stage occurs in which nuclear and cytoplasmic elements are mingled together.

So too every somatic cell, when about to divide, undergoes a process of " Entdifferenzierung " or de-differentiation.* The cell division which follows provides in both cases, the opportunity for variation among cells, or cell parts and through the selection of fitter units for adaptation of the " Indirect " or the " Direct " kind.

Thus, for the purpose of the present enquiry we may think of a Use-acquirement as that change in the structure and function of a cell which is brought about by *direct* response to Environmental influence.

THE NEURO-PSYCHIC RESPONSE.

I shall now consider the response made by the nerve cell or neuron, the neuronic arc, and the brain as a whole, to environmental change, as examples of a Use-acquirement.

In the first place, the highly specialised adult nerve cell undergoes neither de-differentiation nor cell division. This is a suggestive fact, when we remember that it is the nerve cell or cerebral neuron which exhibits the faculty for making use-acquirements in the highest degree. On the other hand the nerve cell is pre-eminent among somatic cells in possessing the capacity for rapid re-construction and re-arrangement of intra-cellular elements. The whole life of the functionally active nerve cell is a process of direct adaption to changes in environmental stimuli The fact that indirect adaption is associated with cell division and regeneration, and direct adaption with functional activity, suggests that both kinds of adaptive response depend on variation and selection acting on units of a different order.

It is probable that, among the many responses made by cells of different types during individual development, the response made by the cerebral neurons in man, and in some of the higher animals, affords the best example of a response made to the use stimulus. It constitutes,

* See Strangeway's Tissue Culture.

in fact, a true use-acquirement. In confirmation of this view we find no evidence of any struggle for existence, or cell destruction, or of cell regeneration among nerve cells, at any rate when the neural response falls within the limits of normal functional activity. The functionally active nerve cell does not die, nor undergo cell division, at any rate in the human subject during adult life. It is the undifferentiated neuroblast, and not the highly complex neuron, which divides and multiplies during fœtal and early post-natal life.

In fact it is true to say that in proportion as the neuron develops the capacity to respond *directly* by use-acquirement, it loses the capacity to respond *indirectly* by cell division, thus markedly differing in this aspect, as we shall see later, from the muscle cell.

This absence of destruction and regeneration of nerve cells, during adult life, is inconsistent with the selection of more favourable varieties of nerve cells as an explanation of neural adaptation, but it is consistent with the selection of, and the favourable re-arrangement of nerve cell parts, as being the real process by which neural adaptation is brought about, and by which individual nerve cells make use-acquirements.

But if this conception of the nature of a use-acquirement is sound, then there should be some evidence of the presence, during neural adaptation, of the three conditions, viz., struggle or competition, destruction or removal, and regeneration or reconstruction, (not among individual nerve cells), but among the intra-cellular elements of which nerve cells are composed.

Such evidence does exist.

Hodge, Gustav Mann, and others have recorded the effect of fatigue and prolonged stimulation in reducing the size and number of the Nissl bodies in nerve cells. The effect of effort is to use up, the effect of rest is to restore the number, size, and integrity of these intra-cellular units.

Mott, Marinesco, and others have also shown that degeneration in the neuron, produced by division of the axon, affects these chromophilous bodies, or Nissl granules. But where competition for nourishment exists among units, and where units, intra or inter-cellular, are being destroyed and regenerated, then, as we have seen, the conditions are present which favour the selection of favourable varieties among the units concerned.

As we descend the zoological scale, we find an extensive fibrillary continuum between nerve cells associated with the more simple sensori motor responses. On the other hand, structural discontinuity is associated with increasing complexity of sensori-motor response.

Ramon-y-Cayal and others have shown that both the phylogenetic and the ontogenetic evolution of the sensori motor neurons takes place by an increase in the complexity of cell processes, as well as by an increase in the number of cells.

It will perhaps be objected that Nissl granules and nerve fibrils represent artifacts.

It is no doubt true that all that can be seen under the highest powers of the microscope, in the cytoplasm of the living nerve cell, are numbers of refractive granules exhibiting movement in a clear colloidal medium. But artifacts have pre-existing phases in the living substance in which they are formed, and when differences, in modes of cell activity and metabolism, are found in constant association with differences in the artifacts which follow them, then such artifacts become important as indicating previous events and pre-existing conditions. For whether with Macdonald we regard the neural discharge as associated with the translation of electrolytes in a living medium, or with Macullum with re-distribution of potassium and other ions during differences in surface tension, yet in either case qualitative and quantitative changes in a living medium provide the material basis for the constructional and functional changes which constitute neural adaptation.

But there is another side to this picture of the life of the nerve cell.

The cerebral neurons, in certain areas of the brain, are characterised by the development of fine dendrites, and it is the juxta-position of the terminal dendrites of one neuron with those of another, without perhaps actual structural continuity between the two, that provides for the orderly transmission of the neural wave across the synaptic field, and so for the physiological continuity of the neuro-psychical message from one neuronic arc to another. The making of neural use-acquirements is thus associated, not only with the laying down of nerve paths in the individual nerve cell, but also with the establishment of neural routes between one neuron and its neighbours.

We know but little about the way in which communication is established between the terminal tendrites of one cell and those of another across the synaptic field, but we can gain some notion of the accuracy and delicacy of the process by observing the wonderful way in which the dendrites of one leucocyte establish " end on " contact with those of a neighbouring leucocyte, in a living film of emigrating cells.

The accuracy of the process is marvellous when we remember that these terminal processes lie at the extreme limits of visibility under the highest powers of the microscope, and that the contact established is " end to end " and not " end to side," or " side to side," in an accidental manner. ("The Leucocyte in Health and Disease," fig. 4 and 5. Lewis & Co., London, 1924).

Now although actural structural continuity between the terminal fibres may be *absent in the case of neurons, yet it would seem likely that there must be some orderly approximation between them which favours the passage of the neural wave in one direction rather than in another across the synaptic field, and there is the further known fact that the wave travels in one direction only, *i.e.* from

* Ransom—" The Nervous System."

sensory to the motor side of the nervous system. But this system of arborisation—these dendrites, represent parts of the individual nerve cell, and they must share in that variation and selection of intra-cellular parts which, as we suggest, provides the material basis for the making of use-acquirements during the neuro-psychical response.

In thus speaking of discontinuity in the relationship between the neuronic elements of which the vertebrate central nervous system is built up, I am not unmindful of the work of various observers (*e.g.* Dr. O. W. Tiegs, Sect. D, Brit. Assoc, 1928, and Miss F. Ballantyne on the Nervous System of Lepidosiren, Trans. Roy. Soc. Edin., Vol. 53, R3.), which suggest that though the forward, or uni-directional flow of the neural wave must depend on unequal resistance, yet discontinuity may not take the form of a visible gap in the neuro-fibril chain.

Thus by reconstruction and re-arrangement of intra-cellular parts of the body of the nerve cell, and by alteration and re-arrangement of nerve paths and termini in its arborisation system, the neuro-phychic response undergoes development and integration, without any destruction or regeneration of neurons as such, and in this way much waste of valuable material and energy is avoided, when use-acquirements of a neuro-psychical kind are made.

THE NEURO-PSYCHIC RESPONSE MADE BY THE BRAIN AS A WHOLE.

We find further evidence of this intra-cellular variational and selective process when we come to study the response made by the nervous system as a whole.

The great characteristic of the human mind is *Educability,* or capacity to profit by experience.

The making of neuro-psychic acquirements, that is the learning of new ways of thinking and acting, the formation of habitual modes of conduct in response to new sensory impressions, is really a process of selection—, of

trial and error. It consists in the selecting of one out of many possible modes of reaction in response to environmental influences. The neuro-psychic response on its physical or neural side means, in short, "nerve path making." In proportion as any nerve path is habitually traversed, it becomes easy and direct, and in proportion as competition, and consequent resistance, ceases among the intra-cellular elements in the neuron, the response becomes automatic, and may indeed cease to be represented in consciousness. The fact that the *direct* or "post experiential" mode of response has come to overshadow and supplement the *indirect,* or "pre-experiential method," in the higher centres of the human brain, largely depends on the fact that the units concerned in the process are of the intra-cellular kind.

In the brain of animals, even those of the higher species, the pre-experiential mode of response is still predominant. We see this in the larger part played by reflex and instinctive behaviour in their lives. The neural paths are for the most part laid down before birth. The heredity basis on which educability, or the capacity to learn by experience rest, depends on the other hand, partly on the number of cerebral neurons present, and partly on their capacity to initiate intra-cellular changes in response to fresh environmental stimuli, and partly on the establishment of a complicated system of nerve paths between the different nervous and neuronic arcs concerned.

We have then arrived at this point.

The absence of destruction and regeneration of nerve cells during the making of the neural response in post natal life in man, shows that the neural adaptation cannot be due to the selection of favourable varieties of nerve cells and the rejection and death of those less fitted to survive under the new conditions. The further fact that the neuron does not undergo structural or functional development, or become "adapted," in the absence of the use stimulus, shows that the adaptive change is not

wholly the outcome of growth tendencies set going in the nerve cell by nutritional stimulation alone. We know, for instance, that the brain cells of the child must be stimulated into activity by sensory impressions from the outer world if they are to undergo normal development.

Further the evidence is conclusive against the direct influence of the environment as the sole cause of efficient neural adaptation independently of inherited nerve cell potentiality of development. Hence we are driven to the conclusion that the neuro-psychic response, like other adaptive responses, is the result of an interaction between environmental stimulus and cell potentiality. The question remains—what part does each of these two factors play in the response made?

If the above conception of a use-acquirement, as a process of intra-cellular variation and selection be true, then the part played by the nerve cell must be that of initiating different modes of *intra*-cellular activity, and the part performed by the environmental stimulus that of selecting the particular mode which is most responsive to the stimulus on the one hand, and the most conducive to the life of the cell on the other.

It may be useful to consider for a moment any alternative possible ways in which the neuro-psychic response could be established, other than that by such a process of variation and selection among intra-cellular units.

We have already seen that the making of mental use-acquirements—*i.e.* post experiential neuro-psychic adaptation, does not depend on innate cell potentiality alone, (learning does not take place in the absence of teaching), nor does it depend on environmental stimulus alone, for without some capacity of response on the part of the cell, environmental stimuli are inoperative. The extent and accuracy of the mental acquirements made are the outcome of a reaction between both factors, and it is difficult to conceive of any way in which the individual neuron can contribute to the result, except by providing a number of alternative modes of intra-neuronic activity, and

alternative inter and intra-cellular paths, from among which the sensory stimulus, flowing in from the environment, selects or uses the one which is most suitable and most easily traversed, for it is by repeated use that any particular path is established. The environmental stimulus does not make its own path through the nerve cell or neuronic arc independently of any reaction on the part of the nerve cell, neither does the nerve cell offer to the entering stimulus one single, or wholly predetermined route alone, at any rate in the case of post-experiential adaptation. The predetermined route is in fact characteristic of the instinctive re-action and of the pre-experiential mode of response.

Thus we must, I think, fall back on the explanation now offered—namely, that of variation among intra-cellular units, and the selection (by the environmental stimulus) of that particular arrangement of units, *i.e.* the nerve path, which offers the line of least resistance. Moreover it is important to realise that the *direct* form of adaptive response, as exemplified by the neuro-psychic reaction, has grown and developed out of the *indirect* intercellular mode which it now partly supplements in the human brain. Voluntary or rational behaviour, which rests on alternative modes of thought and conduct, has come to overshadow instinctive predetermined conduct.

The simple reaction becomes the conditioned reflex of Pavlov.

Moreover this change has been brought about with great economy in a highly complex, living material, and in neuro-psychical energy, the most valuable, and speaking physiologically the most expensive, of all known forms of energy.

At this point I want to make it clear that we are not now concerned with the mode of origin of the modifications of the *intra*-cellular elements which form the basis of use-acquirements. For our present purpose it does not matter whether these intra-cellular variations depend upon some

"Effort" on the part of the cell, or on "Habit" or "Inherited Cell Memory" in Hering's or Samuel Butler's sense, though it seems difficult to explain any departure from habitual modes of activity on any theory of cell memory.

The real question now before us is whether such variations occur in many directions in the same cell independently of environmental influence, or whether they take place in one direction only, which has, or may have, some reference to a corresponding outside stimulus. The evidence here presented seems to show that the former is the true answer.

Thus, whether we study the method of the response made by the individual neuron, or by the neurotic arc in co-operation with other neurotic arcs, or whether we study the response made by the brain as a whole to the stimuli which reach it from the receptor organs, we find that the method is one of "trial and error." It consists in the selection of one, out of a number of alternative modes of activity, and the choice of one out of many possible nerve routes, both through individual neurons and between different neuronic arcs.

It represents in fact a process of variation and selection among intra-cellular units. *The Philosopher James Ward defined "Experience" as the process of becoming Expert by Experiment—that is by a process of trial and error.

In this address I have endeavoured to describe in physical or neural terms the change which takes place in a nerve cell when it "becomes Expert through Experiment" *i.e.* when it makes a use-acquirement or in other words when it learns by experience.

If we could only understand her message, Nature would have much to tell us, not only of the wonderful way in which this *post*-experiential method of use-acquirement in the individual has developed out of the older *pre-*

* J. Ward—Heredity and Memory. Cambrian Press, 1913.

experiential instinctive method,—but also—how greatly the pace of the evolutionary process has been quickened by the integration of numerous and diverse capacities of pre-experiential adaptation, into one unified capacity of adaptability and educability.

She would tell us how, and in what way, many different pre-experiential forms of adaptation to diverse environmental stimuli, in different animal species, at different periods of evolutionary history, had been gathered together and were now largely represented in one unified capacity, in one organ, the human brain, and how this allows of still further adaptation to an ever widening environment in space and time. There is a hidden truth in the saying " Use is Second Nature."

The fact that this peculiarly human capacity of educability, or capacity to learn by experience, has grown out of the more primitive mode of pre-experiential response, and that it has been evolved by the same process of variation and selection which has brought about the process of racial evolution is a very suggestive one.

The earlier stage of human evolution, when man was emerging from the ape like, and passing into the human form, seems to have been a period in which this growth of educable capacity was most rapid. In other words it was at this particular stage in human evolution that the post-experimental adaptive response possessed its greatest survival value.

On the other hand judging from the little we know of human development during the historic period, no great increase in this capacity of learning by experience seems to have taken place in the human brain. This slowing down in the development of innate mental capacity during the historic period may have been due to a number of causes, among which loss of survival value under modern conditions of so-called civilisation is probably one of the most important. Innate capacity is not being hereditarily transmitted under modern social conditions to the same extent as in former times.

There are, however, grounds for believing that a quickened rate of increase will again take place when modern man, under the stimulus of economic and social pressure, and aided by the growth of biological and genetic knowledge, begins to exercise that conscious control over the production of both quality and quantity in the human life of the future, which was exercised by natural selection in the past.

MUSCULAR HYPERTROPHY.

We must now proceed to compare the neuro-psychic response with other responses made by the individual cell or the organism to the " use " stimulus.

Muscular Hypertrophy, the result of increased muscular effort, is a use-acquirement, but it differs in its nature and mode of origin from the use-acquirements made by nerve cells. When a voluntary muscle enlarges as the result of increased effort, the increase in size is due to an increase in the number of muscle cells and not to any variation in the intra-cellular elements of which those cells are composed. The increased stimulation produces, not a modification of intra-cellular structure and function, but a repeated cell division of muscle cells, not probably of the fully developed, highly specialised cells, but of the younger, less differentiated cells which grow into striated muscle fibres. Apparently muscle cells vary in size *i.e.* in diameter in different muscles in the same individual (see Nature, Ap. 2, 32).

The Blacksmith's well developed biceps muscle no doubt represents a use-acquirement on the part of the Blacksmith, but it does not represent a use-acquirement on the part of each muscle cell which enters into the formation of the hypertrophied biceps. The same is true of the heart muscle in cardiac hypertrophy, and of the non-striated muscle fibres of the uterus during pregnancy.

Use-acquirements are thus brought about in different ways, by change and reconstruction of *intra*-cellular

elements as in the nerve cell, or by increased cell division and cell-multiplication as in Muscular Hypertrophy.

The difference in the two cases lies in the kind of units, intra or inter-cellular, concerned in the response. Thus we see how necessary it is to judge of use-acquirements, not so much by their mode of manifestation in the individual organism, as by their mode of origin in the individual cell.

We must also remember that though the use- acquirements, made by somatic cells, are not normally transmitted, as such, to offspring, yet, at any rate in the human subject, the cell potentiality, the capacity to vary intra-cellularly, in the case of the nerve cell, and the ability to undergo repeated cell division in the case of the muscle cell, do represent innate germinal characters, and as such, are hereditarily transmitted to offspring.

It is also important to recognise that the making of use-acquirements is a very different problem from that of their *transmission,* when made, to offspring.

The modern conception of the organism as one whole, with activities starting within, or influences flowing in from without, is thought by some biologists to afford an adequate explanation of the way in which the germ cells may become modified in the genetic sense. This idea seems, however, to be somewhat lacking in definiteness, and we still await reliable evidence of the existence of some adequate mechanism by means of which, somatic acquirements can be handed on to the germ cells and thus transmitted to offspring.

THE RESPONSE MADE BY EPITHELIAL CELLS TO ENVIRONMENTAL CHANGE.

The new characters acquired by epithelial cells, when for instance a portion of mucous membrane is grafted on to a skin surface, that is from a moist to a dry environment, also provide a good example of the making of a

use-acquirement from the point of view of the individual organism.

In such a graft the young, undifferentiated epithelial mother cells, give rise to daughter cells, which lose their mucous secreting capacity and their columnar shape, and become squamous cells arranged in a stratified layer like epidermal skin cells. The older cells meanwhile disintegrate and are shed.

The same kind of change in the opposite direction also happens when epithelial skin cells are transplanted to a mucous surface, but in neither case does a full reconstruction of the skin tissue or mucous membrane take place. Neither sweat glands, nor hairs, nor mucous glands are restored.

Thus this case turns out on examination to depend on an inherited capacity on the part of the younger, undifferentiated cells, to develop along one or two alternative lines of growth, according to the nature of the environmental stimulus, that is according to whether the climate is an aerial or an aquatic one.

The same result can be brought about by transplanting the ureters in a case of extroverted bladder. After the flow of urine has been diverted and the surface is kept dry, the red mucous membrane becomes transformed in time into a skin surface.

This dual capacity on the part of the cells which form the inner and outer coverings of the body, is of peculiar interest because it is probably associated with the change from an equatic to an aerial environment, which characterised the migration on the part of certain primeval animals from the sea to the land.

We see the same process going on in an abbreviated form in the production of the vernix caseosa in the human fœtus when, in the later months of pregnancy, it is beginning to develop a skin surface while still living in the watery environment of the liquor amnii.

It is probable that this inherited capacity of development along one or two alternative lines of growth,

according to the nature of the environment, may also be the explanation of the changes undergone in the skin characters of certain amphibians, when removed from an aquatic to an aerial environment, and vice versa, and possibly also to the altered characters acquired by alpine plants when transplanted to a lowland environment, as suggested by Walker.

Here then the change in character undergone by skin cells, when submitted to environmental change turns out on examination to differ from the true use-acquirements made by the nerve cell. It is accompanied with cellular destruction and regeneration, and it is inter-cellular rather than intra-cellular in nature. It is in fact an *indirect* and not a *direct* response to environmental change.

In a paper on "Histologic Changes in the Nasal Mucosa following Experimental Variations in Ventilation and Exposure" (Proc. Mayo Clinic, Dec. 30, 1931), Anderson Hilding has shown that, when one nasal opening has been closed (in the dog) for some months, the Epithelium covering the membrane on the extra ventilated side of the septum, becomes squaruous and stratified, or skin-like in character. In the same way the lining of a frontal sinus which had been freely exposed to the air for 85 days took on a skin-like character.

CANCER REGARDED AS AN ADAPTIVE RESPONE.

We will now compare the response made by the normal epithelial cell with that made by abnormal or cancer cells of the epithelial type.

The three chief kinds of environmental change with which the abnormal cancer cell, like the normal epithelial cell, is brought into contact are:—

Changes in the

1. The nutritional factor.
2. The neighbouring cell factor.
3. The external world factor.

(1) The Nutritional Element.

While the nutritional element is, of course, common to all cells, normal and abnormal, the cancer cell, as Warburg and others have shown, differs from the normal cell in being less dependent on an abundant oxygen supply for its nutrition and growth.

(2) The Neighbouring Cell Factor.

With regard to the neighbouring cell factor, this also is common to both normal and abnormal, or cancer cells, though it operates differently in the two cases.

It is now forty-five years since the suggestion was put forward (see *Transactions of the Leicester Medical Society,* 1886), that the stroma and the connective tissue capsule of a glandular organ, like the kidney or liver, is the outcome of a reaction between the mesoblastic cells which form the limiting membrane, and the epithelial glandular cells derived from the epiblast. It is indeed only by some such process of contact adjustment and response, between cells and cell groups of different types and function, that we can explain the orderly growth of the tissues and organs of the multicellular organism. In the earlier stages of the developing fertilised egg cell we know that the influence of the neighbouring cell factor is very great. The facts derived from a study of tissue culture in vitro also confirm this view of the importance of the neighbouring cell factor.

Drew, for instance, has shown that the effect of adding a sub-culture of connective tissue cells to a pure culture of epidermal cells from the same organism, is to cause these latter cells to re-differentiate and to assume the prickly and keratinous stratified character, peculiar to skin epithelium.

The same effect in the way of increased differentiation is seen in a culture of mammary mouse cancer cells to which some connective tissue cell culture has been added.

In cancerous growths this delicate relationship between the surrounding tissue cells and the epithelial cancer cells may be profoundly changed. If we may so express it, the object of the cancer cell is so to stimulate the reaction made by the surrounding connective tissue cells, as to ensure the growth of a nutritional scaffold rather than an enveloping limiting capsule. Both the clinical and cytological findings in the case of malignant tumours afford conclusive evidence of the importance of this reaction. See J. J. M. Shaw "On the Etiology of Cancer—*Lancet,* Jan. 30, 32. See also the 10th Report of the Imperial Cancer Research Fund, 1932.

3. The External World Factor.

The normal epithelial cell of the glandular type and the cells covering a skin or mucous membrane surface have one side or area of the cell in contact with its nutritional base and the other exposed to the external world. From its nutritive base the cell derives nourishment and is thus enabled to elaborate the secretive or protective material which is delivered on to the free surface.

With the cancer cell it is otherwise. In fact it is true to say that the degree to which the epithelial cancer cell loses the power to elaborate normal selection, or to deposit protective material, is an index of its malignancy. With increased activity in differentiation and normal function goes increased activity in cell division and cell reproduction. In other words what the cancer cell loses in capacity of response to the "use-" stimulus it gains in capacity of response to the stimulus of nutrition.

Although Cramer, Warburg, and others, as quoted by Shaw (see above) are no doubt right in regarding capacity for growth as a property inherent in the cell, yet it is legitimate to speak of an inverse relationship between growth and function. Such a relationship exists, and

varies in cells of different type as we have seen in the case of the nerve cell. But it may be asked—Do we gain anything by trying to describe Cancer in Biological terms as an adaptive response made by the cancer cell to altered environmental conditions.

It is true that by so doing we do not gain any further information about the nature of the environmental influences to which that response is made, in other words about the immediate cause or causes of cancer. It does not tell us whether that influence is a virus in combination with a specific factor of cellular origin, as Gye and Purdy think, or whether the carcinogenic agent is a chemical substance, or whether it is some form of radiation.

On the other hand such a description is I think useful. It is an attempt to form a mental picture of what may be happening in the cancer cell, when it is exposed to environmental change, and it is with what happens *in* the cell and the conditions under which this change takes place, that we are now specially concerned.

First, as to the nature of the change in the cell. This must be some alteration in the mode of cell metabolism, one which affects not only the cytoplasm, but also the nucleus, or at any rate the chromosomal heredity mechanism of the nucleus, because, as we have already seen, the newly acquired mode of cell life is transmitted to the cell descendants of the cancer cell, though not, as far as we know, in the human subject at least, to offspring.

The conditions under which the response is made, at any rate in their earliest stages, include cellular destruction and cellular regeneration. The condition of chronic irritation which precedes or accompanies malignancy, represents a process in which many generations of epithelial cells are formed and perish before a cell, or a group of cells arises, which are more fitted to survive and reproduce themselves under the new conditions.

This means that the units concerned in the variational and selectional process associated with malignancy, are inter rather than intra-cellular. The units concerned are cells rather than cell parts, unlike the case of the nerve cell.

But in thus speaking of cancer as an adaptive response, made by certain cells of epithelial type to environmental change, I do not mean to imply that the cancer cell makes any new use-acquirement in the sense that the nerve cell or the normal epithelial cell does, when subjected to environmental change.

As has already been pointed out, the response made by the cancer cell involves a *loss* of functional capacity, a lessened ability to respond to the use-stimulus with an increased capacity to respond to the stimuli of nutrition and cell reproduction.

In fact, the response made by the cancer cell resembles that made by cells and organisms which are embarking on a parasitic mode of life.

So far I have spoken only of the response made by the cancer cell of epithelial type.

That made by the cancer cell of the connective tissue type, *i.e.* of mesoblastic origin, presents somewhat different features, though both are alike in showing a lessened functional and an increased reproductive capacity. In Sarcoma we find less evidence of cellular destruction and regeneration. The earlier age incidence of Sarcoma and its direct relationship to previous injury point to a direct rather than to an indirect form of response.

Nor have I discussed the reaction made by the cells which form the so-called benign tumours.

For a further consideration of Blastomatosis in general as an adaptive response, I must refer my readers to articles on Cancer published by myself in the *Lancet* of August 5th, 1911, and to articles by Mr. T. C. Clare, F.R.C.S., and Dr. Payne, published in the same Journal, 1920 and

1928, which discuss the cancer process from our present point of view.

*Also to the experiments of Cramer, Payling Wright, and Mottram on the viability of Cancer cells when exposed to injurious environmental conditions such as freezing and thawing, a high concentration of O or Co_2 etc., also to the articles on the Œtiology of Cancer by J. M. Shaw (*Lancet,* Jan. 30 and Feb. 6, 1932).

In thus describing Cancer as an adaptive response on the part of certain cells to environmental change, we may of course ask whether such a conception of the Cancer Process throws any light on the more fundamental idea of the organism as one " whole."

That is to say, does such a view of Cancer help us in any way to decide in favour of the "particulate" or "cellular" as against what is known as the " organismal " theory of evolution and development?

It will at any rate be admitted that, in the case of Cancer, certain cells, or cell groups, break away from the control which is normally exercised by the organism over the activities of normal cells.

Cancer cells pursue a new line of growth and reproduction of their own, which is no longer subservient to the welfare of the organism of which they are component parts.

Now be have seen how cancer cells, while losing their capacity of response to the use stimulus gain in capacity of response to the stimuli of nutrition and reproduction, until finally they become established along a parasitic mode of life.

The question is, how does it come about that the organism which normally functions as a whole, fails to control this aberrant life process in these subordinate but anarchic component units.

Is it the fault of the organism, or of the cancer cells themselves, that these normal and aberrant cells arise and

* Dr. J. A. Murray, 1930-31, Report Imp. Cancer Res. Fund.

flourish in the cell community which represents the organism, or are both partners responsible for the loss of co-operation and control?

If the organism is responsible, then it would seem that the " organismal " theory of development does not cover the exceptional case of Cancer. If, on the other hand, as seems more probable, it is the aberrant cancer cell which starts the revolt, then the behaviour of the epithelial cell while on its way to become a cancer cell, becomes a matter of fundamental importance.

It is at this point that the question arises, whether the change from the normal to the cancerous mode of life represents a " use-acquirement," *i.e.* a direct adaptive response on the part of the cancer cell to changed environmental conditions, or whether it is due to some mutation arising in a so-called spontaneous manner in the epithelial cell before it becomes a cancer cell. As I have endeavoured to show, an examination under the conditions under which Cancer arises throws some light on this point. The fact that the response is accompanied with a high mortality rate among would be cancer cells, together with a still higher cell regeneration rate, suggests that the response is probably *indirect* in character and is concerned with inter-cellular rather than with intra-cellular units.

It is at any rate true that the new mode of cellular activity is transmitted to the cell descendants of the epithelial cells which become cancer cells, and that it is not normally transmitted to the germ cells and so to offspring.

This means that the change becomes incorporated in the nuclear heredity mechanism of the epithelial cells which make the change, or in other words become cancer cells.

Thus it would seem that the adaptive response on the part of certain cells or cell groups to environmental change, which we call " Cancer," fits more readily into the particulate or cellular than into the organismal theory of evolution and development.

THE "USE-ACQUIREMENTS" MADE BY UNICELLULAR ORGANISMS.

It will be useful at this point to compare the adaptive responses or use-acquirements made by certain unicellular organisms with those made by epithelial cells to environmental change.

The researches of Cohn, Balbiani, Dansyz and others have shown that some infusoria and other protozoa become adapted to nutrient media of a different chemical, and even a toxic, composition by a gradual process of acclimatisation and, if this process is sufficiently gradual, it may not involve the death of the organism concerned.

Dallinger showed that the same thing may occur through change in physical environment. He gradually acclimatised infusoria to a water temperature of 23°c without causing their death, and later to a temperature of 70°c.

Metchnikoff says, in regard to Davenport's and Neal's experiments on the acclimatisation of Stentors to four times the lethal percentage of mercury chloride, that the immunity cannot be attributed to the selection of those stentors which possess a natural resistance to the sublimate, but that it is the result of a gradual and direct chemical influence on the protoplasm of the stentors which, once acquired, enables all the animals to survive doses, lethal to unacclimatised control individuals.

The experiments of Stahl are also suggestive from the point of view of acquired immunity.

Plasmodia of myxomycetes were habituated to, and finally attracted by, solutions of glucose of a strength formerly injurious to them, and towards which they previously manifested negative chemio-taxis.

Metchnikoff brought about the same result with arsenical solutions.

In this case, as with Dallinger's infusoria, adaptation appeared to be associated with hydrolytic changes. The

protoplasm of the adapted individuals contained less water than that of the controls.

Thus the evidence suggests the possibility that adaptation to environmental change can take place in some unicellular organisms by a process of use-acquirement, unassociated with the death of any of the organisms exposed to the change, and therefore independent of any selection among the organisms concerned.

Possibly a similar explanation may also apply to certain pathogenic organisms of the coccal or bacterial kind. Further the above observations go to show that in the case of certain unicellular organisms the variational and selectional process which underlies the response, takes place among intra-cellular units, and the change is thus transmitted to the cell descendents of the cells which make it. It becomes an inherited character.

In a demonstration to the Members of the Genetical Society at the Lister Institute on March 17th, Dr. M. Robertson showed the effect of Acriflavine and Radium in bringing about modifications in the Protozoan (Bodo Caudatus).

The change in certain characters exhibited by strains of this organism, after such treatment, was attributed to:

(*a*) A selectional process leading to the elimination of certain individuals and

(*b*) Modifications brought about in the bodies of other individuals, the result of environmental influence, whereby a more resistant or acclimatised strain became established.

If this be the true explanation, then the establishment of a "Fast" or resistant strain in this organism is the outcome of both *direct* or intra-cellular, and *indirect* or inter-cellular variation and selection.

THE RESPONSE MADE BY BONE CELLS.

The response made by osteoblasts to changes in the

stimuli of stress, pressure, and muscular strain, is worth a short consideration.

At first sight, many persons, even physiologists, would be inclined to think that the inherent tendency, derived from ancestral inheritance, of the bone cells which enter into the composition of the human fibula for instance would lead those cells to grow and arrange themselves in the same " fibular " manner, even when transplanted to other situations in the limb, or body, where the stresses and strains produced by superincumbent weight, position, and surrounding muscles act in a different manner.

But experiment shows that this is not the case.

Some twenty-five years ago, in order to restore the usefulness of the limb in several cases in which the diaphysis of the tibia had been completely destroyed by osteomyelitis, I transplanted the fibula in the same limb to the situation formerly occupied by the tibia and attached the transplanted bone above and below to the tibial epiphyses which had not been destroyed by the necrotic process.

X-Ray photographs of these limbs taken twenty-five years later show that the transplanted fibula had, in each case, been transformed into a tibia. The fibula osteoblasts, under the new conditions of stress and strain, had grown and arranged themselves in a way which had produced a bone shaft which in size, shape, and conformation could not be distinguished from a tibia.

Here then we have, when judged from the point of view of the individual organism, a use-acquirement.

But here, as in the case of epithelial cells when transplanted to new conditions, the true explanation of the adaptive response must, I think, be found in the capacity possessed by osteo-blasts, and apparently bone cells generally, to respond in an adaptive way to the peculiar stimuli of stress and strain and muscular pull to which they are exposed when under the influence of changed conditions, as in the cases now mentioned. When the result is to the advantage of the organism as a whole, we

call it a use-acquirement. It is well also to remember that in bone formation, and bone architecture, the result depends on a balanced combination of cell formation and cell removal, on the laying down of bone and calcium salts in one area, and the removal of the same materials at another site of the developing structure. In this connection we recall Goethe's law of balance and correlation. Goethe said "In order to spend on one side Nature is forced to economise on the other."

Those who may be interested in the response made by bone cells will find a description of these and other cases of bone transplantation in Sir Arthur Keith's book—"Menders of the Mained." (Hodder and Stoughton, 1919). Page 273, etc. In this connection also reference must be made to the interesting observation of Dr. Robison at the Lister Institute on Bonegrowth in vitro.

THE ACQUIRED IMMUNITY REACTION.

We must now compare the acquired immunity reaction with the neural, muscular, and epithelial responses from the same point of view. From the point of view of the individual, the acquired immunity reaction fulfils all the conditions of a use-acquirement. It is the result of environmental change, in interaction with cell activity, and it is not transmitted to offspring. But when we consider it from the point of view of the defensive group of body cells, including those of the endothelial, reticular type, which are concerned in the reaction, it assumes a more complicated character.

A word of Explanation must first be given.

The immunity reaction as a whole falls into two main divisions—the *natural* immunity, which comes about through the selection of more resistant individuals during racial experience of disease, and the *acquired* immunity which results from the exercise of the capacity of recovery during individual experience of disease. It is with the

non-transmissible acquired form that we are now concerned.

In the first place the immunity to subsequent invasion by the same disease organism or virus, varies in completeness and persistence in different diseases. Thus the immunity which follows recovery from a localised staphylococcal infection accompanied with suppuration, may be partial and evanescent, while that which follows recovery from an attack of one of the virus diseases, like Small-pox of Measles, is usually complete and lifelong.

What is the meaning of this difference?

Does it depend on the fact that the reaction in the localised form of infection, which is accompanied with suppuration and cell destruction, takes place only among cytoplasmic elements, while in the more generalised form of infection it affects the nucleus and the nuclear heredity mechanism of the cell. If this be so, we can more easily understand why the acquired immunity which follows recovery from a localised infection may be incomplete and evanescent, while that which follows recovery from a generalised infection, due to a virus disease, may be complete and lifelong. In the latter case, the immune or resistant character is transmitted to the cell descendants of the cells belonging to the defensive group of body cells.

Now in order to ensure transmission to cell descendants, the virus or infecting agent must affect the nuclear heredity mechanism, probably in somewhat the same way as that by which mutations are brought about by X-Ray radiation. However this may be, the occurrence of cell destruction and cell regeneration which accompanies the localised form of infection, and the apparent absence of cell destruction in the generalised form, point to the fact that the acquired immunity reaction is a composite reaction, into which the *inter* and *intra*-cellular modes of response both enter.

Thus a critical examination of the immunity response as a whole leads to the conclusion, that both the natural and the acquired form are the outcome of a process of

variation and selection occurring among units of a different order in each case.

In the racially acquired, or so-called natural form, these units are the germ cells, and those cells ultimately survive which develop into more resistant, less susceptible individuals. In the individual or acquired variety, the units are the body cells which react to, and overcome, or are overcome by, the infecting agent, and in some cases the intra-cellular elements of which phagocytes and other types of defensive cells are composed.

Judged from the point of view of the individual organism, adaptation to disease is a " use-acquirement," while judged from the point of view of the units (cells or cell parts) which become adapted, it consists in a process of *inter* and *intra*-cellular variation and selection.

In an interesting article on Immunity in Virus Diseases, published in the *Lancet* of May 2nd, 1931, Dr. C. H. Andrewes made the suggestion that " the immunological properties of viruses are probably related to the small size and the intra-cellular parasitism " of these agents.

If for *intra-cellular* we substitute *intra-nuclear* parasitism, then the intimate and causal relationship between intra-nuclear invasion and the transmission of the immune state to cell descendents become more readily understood.

The experiments of Muller and other observers have shown that irradiation of the sex gonads of Drosophila with X-Rays is capable of initiating alterations in the gene complexes of the germ cells, which then appear as mutations in the individuals which spring from these cells, and these again re-appear in the next generation.

In somewhat the same way we may suppose that the action of a virus after it has gained access to the nucleus of a somatic cell is to bring about changes in the Chromosomes or genes, whereby the altered mode of cell activity and cell metabolism which represents the immune state (or state of insusceptibility to subsequent invasion by the

same disease virus), is handed on to the cell descendants of the cells which have acquired the new capacity.

The element of minute size in the case of a virus, as compared with the much larger size of a bacterial or coccal organism is important, because it affords a possible explanation of the reason why the virus can pass through the nuclear membrane and affect the heredity mechanism, while the much large coccal or bacterial form can only act on cytoplasmic structures.

The presence of so-called nuclear inclusion bodies does indeed provide direct evidence that the nucleus is in some cases the site of invasion by or is affected by the virus.

To sum up the conclusions which emerge from a critical examination of the immunity response, we find that both the natural, or racially acquired, and the individually acquired form are brought about by a process of variation and selection acting on units of a different order in each case. In the natural immunity which follows racial experience of disease those units are the germ cells, while in the acquired form which results from individual experience of disease, they are (in certain virus diseases at least) the intra-cellular molecular units of which the somatic cells are composed. In coccal and bacterial infections, especially in those accompanied with local suppuration, the variational and selectional process is also partly, perhaps largely, *inter*-cellular.

For a fuller discussion if the immunity reaction I must refer my hearers to an article in the B.M.J. of February 24th, 1912, on the "Immunity Problem and Organic Evolution."

CONCLUSIONS.

In conclusion I wish to anticipate, if I can, some objections of a more general character, which may possibly be raised to the theory of the nature of use-acquirements which I have put forward in this address.

Some may say that, since the Darwinian theory of

variation and selection is no longer regarded by biologists and geneticists of the newer school of thought as a complete and satisfactory explanation of the evolutionary process, therefore the explanation now offered of the nature of use-acquirements, as based on variation and selection among intra-cellular units, must also fall to the ground.

My reply must be that, in common with many others, I regard Darwin's theory of variation and selection, as a main factor underlying evolution, and as still true in the phylogenetic field.

What I have endeavoured to do is to apply the same theory to the ontogenetic field, that is to the intra-cellular as well as to inter-cellular units, in order to explain the way in which use-acquirements are made during the lifetime of the individual organism. The mode of application however, differs from that of Weisman and Roux and other preformationists in certain important points.

If the theory of variation and selection is applicable in the case of racial evolution, and in the struggle between organisms and species of organisms, it is surely worth testing in the intra-cellular field and among the smaller units of which cells are composed.

It is true that we find on closer examination that the only response which satisfies all the conditions associated with a true use-acquirement is that made by the nerve cell and the nervous system as a whole to environmental change.

The neuro-psychic response which depends on the post experiential method of learning is the best, probably the only real example, of a true* use-acquirement.

It is also true that the Darwinian theory of evolution does not attempt to explain the *origin* of these variations or difference between organisms on which selection, natural and artificial, works, but this also applies to intra-cellular as well as to inter-cellular variation and selection.

* True that in the sense that it is Direct and concerned with Intra-cellular units.

Neither does it decide as to the relative part played by environmental influence and cell potentiality in determining the ultimate result.

The problem of " who " or " what " determines which cell or organism, and which mode of intra-cellular activity shall come into existence, and which shall possess survival value, is not limited to the biological field.

Biologists, like physicists, are looking for Clerk Maxwell's " sorting demon." Distinguished Physicists tell us that, in the world of the infinitely little, the world within the atom, rigid determinism no longer holds absolute sway.

May it not be that, in the world within the nerve cell, where physical energy is associated with psychical manifestations, a still greater latitude of alternativism, or " choice " between different modes of intra-cellular activity may be present in even greater degree.*

It is in this neuro-psychic sphere, at any rate, that the element of choice appears to us to play a predominant part in our individual lives.

But still another important question arises from this theory of the nature of use-acquirements here suggested.

So far, we have been concerned only with their mode of origin in the body of the organism or cell. We have not entered into the vexed question whether such acquirements are, or are not handed on, as such, to offspring.

It is true that we *have* shown how in the case, for instance, of the acquired immunity reaction in man, the newly acquired character of " resistance " is transmitted to the cell descendants of the cells that acquire the new character, but that it is not transmitted as such to offspring. The children of parents who have acquired immunity against Small Pox or Measles, are still susceptible to those diseases. This is true in even a more marked degree

* See Sir A. Eddington's " The Decline of Determinism " Nature. Feb. 13, 1932.
See F. A. Sandemann " The Physical Significance of the Quantum Theory." Feb. 13, 1932.

in the case of the use-acquirements made by the nerve cell. The adult nerve cell leaves no cell descendants. What is transmitted to offspring, in lesser or in greater degree, is the potentiality on the part of the nerve cell to make use-acquirements, and in the case of the brain as a whole the capacity to profit by experience. This capacity as we know varies greatly in different individuals.

Whether this applies to all the responses made by other cells of different types still awaits descision.

In regard to this question of transmission to cell descendants, observations on uni-cellular organisms in relation to the surrounding medium, and on the body cells belonging to the defensive group in the response made to the so-called virus diseases, show that the characters acquired by these cells *are* transmitted to cell descendants, though not to the germ cells of the adapted individual. The suggestion is, therefore, that in all such cases, the change in mode of activity, whatever be its nature and its origin, must have reached and influenced the nucleus of the cell, that is to say, it must have become incorporated in the chromosomal, heredity mechanism of the cell in order that it may be transmitted to cell descendants. There is moreover direct evidence that the nucleus *is* implicated in such cases.

In the case of the acquirements made by the nerve cell, we know that the adult nerve cell leaves no cell descendants, and it is tempting to hazard the suggestion that in this case the intra-cellular changes on which the neuro-psychic response rests are limited to the cytoplasm or perikaryon and do not involve the nuclear heredity mechanism. It is at any rate a suggestive fact that such changes are most noticeable in the cytoplasm in the case of the nerve cell.

In view of the marked and often strongly expressed difference of opinion among biologists on the question of the inheritance of acquired characters, a word of caution may not be out of place.

It may be that the results of experiments and observations on certain kinds of animals, for instance on insects and other invertebrates, may not be directly applicable to other kinds of animals, to vertebrates, or to man.

Harrison's observations on Melanism in Moths and Metalinkoff's immunisation experiments on caterpillars of the Beeswax Moth are regarded by some biologists as proving the possibility of producing an immune strain by environmental influence, without the aid of selection.

In Man, on the other hand, the acquired immunity which results from individual experience of certain infectious diseases is *not,* as we have already seen, transmitted to offspring.

Assuming that the environmental influence, the vaccine, reaches the germ cells of the Beeswax Moth either directly, or indirectly by way of the somatic cells, while in the human being no such effect is exercised by the toxins of disease organisms, may not the explanation of the difference be, that the human germ cells are more sheltered against environmental influences than the germ cells of the insect. They are in short more isolated and less affected by the experiences of the organism.

Perhaps then both sides are right up to a certain point, and the differences in the conclusions arrived at, are due to differences in the conditions of the experiments and the observations in the two cases.

If acquired modifications in structure or function are to be transmitted to offspring, then the environmental influences which set them going must reach the germ cells and exercise their effect on the heredity mechanism of these cells.

Hence it is reasonable to suppose that, if the germ cells in different kinds of animals, or in plants, differ in their susceptibility to environmental influences, then the answer to the question as to the inheritance of acquired characters will be different in different kinds of animals or plants.

We know that certain somatic cells do make use-acquirements, and in this Lecture I have tried to show

that they do so by a process of intra-cellular variation and selection.

We also know that, in the case of the acquired immunity reaction in man, the newly acquired " resistant " character is transmitted to the cell descendants of the defensive cells which acquire it, though *not* to the germ cells. But in order to be so transmitted the environmental influence must reach and affect the nuclear heredity mechanism of the somatic cell. The same is also true of the germ cells. Here too, if it is to produce any result, the environmental agent must reach the nuclear chromosomal mechanism, either directly, as in the case of the mutations caused by X-Ray radiation, or by the nutritional channel, *i.e.* by the blood, or lymph, or the body juices, as in the case of the immunised moths. The only difference is, that the cell descendants of the germ cells are not new body cells, *e.g.* leucocytes, or endothelial cells, but new individual organisms.

Everything then depends on whether the germ cells of the animal or plant used in the observation or the experiment are accessible to, or capable of responding to, the special kind of environmental influence employed. This susceptibility may vary in different kinds of organisms, for instance in vertebrates and invertebrates, in insects and in Man.

The unique capacity possessed by Man of modifying his own personality through use-acquirement, especially in the neuropsychic sphere, has probably made necessary or led to a more complete sheltering of the germ cells in the human subject against the direct effects of environmental influences, in order that the variations of germinal origin should not be swamped by acquired modifications, the outcome of environmental influence.

Civilised Man is being called upon to play an increasing part in the control of his own evolution. Seeing that his knowledge is limited, it is perhaps better that he should use his directive power in an indirect, rather than in a direct manner. That is, that he should co-operate with

the other factors concerned in the evolutionary process by promoting the survival of favourable innate variations of germinal origin, rather than that he should transmit to offspring modifications of his own manufacture.

There may well be more possibilities of progress in an evolutionary method, which is concerned with germinal variations of many and different kinds, than in a more restricted method which depends on transmitted modifications, the result of adaptive response in a few directions only.

But a further question still arises. Biologists, since the time of De Vries and Bateson, have recognised two kinds of change in organisms—one made up of the smaller, continuous variations which arise as the result of environmental influences, and the other the larger, discontinuous, departures which constitute the so-called mutations.

It is of course well recognised that the nuclear heredity mechanism, the genes or gene complexes in the chromosomes must in some way be involved in the case of every so-called "spontaneously arising" mutation. It may be that a more delicate and elaborate cytological technique will show that the change, which is associated with the smaller variations due to environmental influences, are confined to the cytoplasm, and do not reach the heredity nuclear mechanism.

Finally we may ask, does this conception of the neuro-psychic response as a use-acquirement, and as the chief, perhaps the only true example of a pure use-acquirement in the human organism, throw any light on the future development of the evolutionary process as a whole.

The fact just mentioned, that this, the chief example of a use-acquirement is associated with the manifestation of neuro-psychical energy in that most wonderful and most complex of all mechanisms, the human brain, is a very suggestive one. It suggests that the rapidly increasing power of controlling the environment to his own ends, which civilised man has acquired, has been rendered possible by the replacement of a pre-experiential by a

post-experiential mode of response, and that this latter method depends on a capacity to make use-acquirements through a process of variation and selection of the *direct* or intra-cellular, rather than the *indirect* or inter-cellular kind. But this growing power to control the environment is of enormous importance, because it affects the agency which determines the type of human organism, and the kind of mental and bodily qualities which shall survive.

It would seem then that in the latest and greatest of the tasks imposed on civilised man, namely that of "changing human nature for the better," by the exercise of control over human breeding, the result will depend on the development of this ability to make use-acquirements, that is on man's capacity to learn by experience.

The chief object of my address has been to show that the making of " use-acquirements " in the neuro-psychical sphere, that is " learning by experience," is the outcome of a process of variation and selection occurring among units of the intra-cellular order.

Although the study of the making of use-acquirements, that is of " learning by experience," throws very little light on the origin of the differences—the variations, or mutations,—on which evolution is based; yet such a study does, I think, support the conclusion that the response made by the individual to environmental influence is, as in the case of racial evolution, the outcome of a process of selection of alternative modes of response.

In other words, ontogenetic development, and phylogenetic evolution, are essentially similar processes, but concerned with units of a different order. In both cases the factors concerned are (1) cell or individual potentiality, and (2) environmental influence, and the reaction between them.

But one more ultimate question still awaits an answer. How far is this, at first sight mechanistic, conception of the way in which use-acquirements are made consistent with the idea of purpose in evolution?

As Sir J. A. Thompson has pointed out in his Riddell Lectures, numerous instances exist, in which organisms appear to act in a purposive manner, but when critically examined these examples are found to depend on the survival of better, and the elimination of less well adapted modes of activity, through the agency of environmental selection.

We do know, however, that the cell or organism makes the " effort " or initiates the variation, while the environmental conditions determine the survival value in each case. Both act as partners in determining the result.

It is also true that, as we pass from the inorganic to the animate, *i.e.* to life and mind, much which seems to us to be mechanistic and pre-determined, gradually becomes apparently purposive and eventually, as in the human neuro-psychic response purposeful, in proportion to its degree of integration.

Moreover the increasing power on the part of (?civilised) man to control his environment to his own ends, becomes of fundamental importance, because it enables man to determine, to some extent, the test of fitness in the adaptational sense.

Thus, in conjunction with the other partner, viz. " innate capacity to initiate variations," man plays his part in determining his own destiny and in the purpose behind evolution. The insignificant individual organism of the primitive stage become the predominant partner of the later age. Both are parts of one unity, one reality.

But this conception of the individual and the environment as partners in the evolutionary process by no means solves the whole problem.

It does not, for instance, explain evolutionary progress, or the realisation of the purpose, if there be purpose in evolution. For it is capacity to make acquirements, and not the acquirements themselves, which is handed on, in varying degree, from one generation to the next.

Further, as far as we can see, it is not the body cells

or those which make the acquirements which hand the acquirements on, except in so far as such cells or organisms function as the hosts of the germ cells of the next generation. It is the germ cells, with their genes and gene complexes, which, in the multi-cellular organism, hand on all qualities, mental and bodily, good and bad. We have already seen that use-acquirements *are* handed on to the cell descendants of the cells which make them, as for instance in the acquired immunity response, but this does not necessarily mean that such acquirements are transmitted to other kinds of cells, or to cells which are not in direct contact with the special environmental stimuli to which the peculiar adaptive response has been made, that is to the germ cells.

Recent research by Muller and other workers has shown that the germ cells can be directly influenced by certain environmental agencies, such as radiation by X-Rays, and probably in other ways, but we still look for reliable evidence of the existence of an adequate mechanism, whereby acquirements made by muscle or nerve cells can be transmitted as such to the germ cells, and so become incorporated in the heredity mechanism.

How then de we stand in the face of our ignorance as to the way in which acquirements are handed on from one generation to another, if indeed they are so handed on? The biological problem of to-day rests on the discovery of the method, direct or indirect, by which acquired variations in modes of cellular activity become incorporated in the mechanism of heredity.

Meanwhile, it is a suggestive fact that, in both racial evolution and in individual development, that which begins in an apparently blind movement gradually becomes purposive and in man purposeful.

Further, this gradual ascent, this passage from seeming determinism to choice and purposeful activity, proceeds *pari-passu,* with the evolution of consciousness both in the

individual and the race: neurosis becomes increasingly associated with psychosis.

We are, I think, justified in adding that this short and imperfect sketch of the way in which use-acquirements are made also points in the same direction.

THE UNIVERSITY OF BIRMINGHAM
FACULTY OF MEDICINE

WILLIAM WITHERING LECTURES, 1932

ON CERTAIN ASPECTS OF HUMAN BIOLOGY

BY

C. J. BOND, C.M.G., F.R.C.S., F.L.S.

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LECTURE III

GENETICS IN RELATION TO PUBLIC HEALTH AND PREVENTIVE MEDICINE

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PREFACE

THE following Lecture was the third in a series of five Lectures—" On Certain Aspects of Human Biology ," delivered in April and May of this year, under the William Withering Trust

Lecture I—On the Genetic Significance of Hemi-lateral Assymmetry in the Vertebrate Organism

Lecture IV—On the influence of Environmental Conditions on the Shape and Constitution of the Red Blood Cell; and

Lecture V—On the Clinical and Biological Significance of the Observations recorded in Lecture IV. These lectures have already been published.

Lecture III—On Genetics in relation to Public Health and Preventive Medicine—now published—contains certain additions to the Address as originally delivered.

LECTURE III

GENETICS

in relation to

PUBLIC HEALTH AND PREVENTIVE MEDICINE.

One of the conditions attached to the delivery of the William Withering Lectures is, that the Lecturer must deal with some aspect of Human Biology.

May I say then, in justification of the subject I have chosen for this Lecture, that in my opinion there can hardly be any aspect of human biology of more importance to the future of our Nation and Race than one which deals with Preventive Medicine and Public Health in the widest sense.

Preventive Medicine, rightly considered, not only seeks to increase resistance against disease, it should also strive to raise the innate capacity, the mental and bodily qualities of our citizens, to a higher level, and to make citizens of the future better adapted to a changing environment in space and time.

But this means "changing human nature for the better," and any such change in the biological sense can only be brought about by the use of biological methods, that is by the exercise of a wise control over, not only the quantity, but the quality of the human life produced.

There are no doubt people who still believe that human nature is quite incapable of any change by human agency, but a large and growing number of our fellow citizens to-day are becoming convinced, that in so far as natural intelligence and innate capacity of response to a varying environment are concerned, the only way of progress lies along biological lines.

They believe, in fact, that human nature, although it has remained much the same through long periods of

time, *can* be changed, and changed for the better, by the wise application of physiological and genetic knowledge to human breeding and human life.

The Past History of Mankind.

The intensive study which has been devoted in recent years to the palæontological record, and the investigation of the steps by which primitive man has emerged from the Simian type, and has assumed the human form, has revealed many facts of great interest.

It has compelled us to push much further back in evolutionary time those peculiarly human characters which seem at first sight out of place in a pre-human stage of existence.

For instance, recent discoveries have led anthropologists to decide that Pekin man, with his markedly Simian structural features, was a tool making, a fire producing, in fact a human, being.

Coincidently with this great extension in past time of what we may regard as peculiarly human achievements, we are also confronted with the absence of reliable evidence pointing to any growth of intellectual or artistic capacity in the human race during the historic period.

In fact, it seems to be true, that, as far as natural intelligence, power of abstract thought, and artistic ability, are concerned, modern man is but little, if at all, superior to his earlier ancestors of some thousands of years ago.

It is true that the social heritage of mankind has grown enormously during the historic period. By his control over natural forces, and by moulding his environment to his own ends, modern man has climbed far up the hill of Progress. In doing this he has been making use of the tool making and inventive capacity, the adaptability, inherited from earlier prehistoric ancestors. But unless steps are taken to ensure the growth of these essential innate qualities, there is a real danger that man's social, will outrun his racial heritage.

But in spite of this lack of growth in innate capacity during the limited stretch of time covered by the so-called Historic Period, we must not ignore the sporadic outbursts of intellectual life in different races, in different parts of the world, at different periods, in which exceptional intellectual and artistic gifts have blossomed into life. Take for instance the small city state of Athens in the Periclean age, where for a few centuries, the proportion of men and women of high intellectual and artistic endowment, and of genius, in a comparatively small population, was probably far higher than in any community of similar size before or since.

But there is also another side to the picture. When it is studied as a whole, the historic period of which we have been speaking, does not represent a dead level of intelligence and achievement. It is a record of uprisings and downfallings, a story of the growth, maturity and decay of civilisations, cultures, and empires, and, as Flinders Petrie has shown, in the case of successive Egyptian dynastries, the rise to power has followed the invigorating influence of admixture with a more virile and a better endowed race.

The chief lesson which a study of the course of human evolution from prehistoric to modern times teaches us, is in fact, the all important part played by biological factors in human history.

But that which is true of the history of mankind as a whole, is true also of the smaller units of which communities are composed. Thus we find that certain families rise or fall in the social scale, according to the natural endowment, mental and temperamental, of their individual members. This rise to maturity and success, with the subsequent fall, seems in many cases to occupy about three generations, or as they say in Lancashire, three generations from " clogs to clogs."

It is interesting, as I pointed out in the Galton Memorial Lecture (1928) to compare this periodicity in the life of the individual with the corresponding cycle in the life

of a nation, and, provided we do not push the analogy too far, such a comparison, as Herbert Spencer found, may be useful.

Thus we are brought back to a study of the biological factors among which perhaps mental and temperamental endowment, and capacity to resist disease and injurious environmental influences, are some of the most important.

Mental Endowment.

First, then, in regard to mental qualities, among which we include not only intellectual, but temperamental and artistic capacities and emotional equipment.

Modern physiological, psychological, and genetic knowledge have now made it abundantly clear, that, for the normal working of the mind, there must be present in the brain of the individual a minimal number of healthy neurons, or nerve cells, capable of harmonious function.

Further, it is this cerebral organ or neural mechanism, together with the possibility of its development under suitable stimulation, which forms the material basis of mental activities. It is this which is transmitted from parents to offspring, and from one generation to the next.

For as Mott, Berry, Branson, and other neurologists have insisted—without a sufficiency of healthy cerebral neurons, mind and mental manifestations become impossible.

We now know a good deal about the laws which control the hereditary transmission of this supremely important cerebral mechanism, which forms the material basis with which consciousness and mental activities are associated.

We know, for instance, on reliable genetic evidence, that mental qualities, though dependent on a larger and more complex system of heredity factors, or genes, are yet subject to the same laws, and follow the same genetic method as that which controls the transmission of bodily qualities.

On this matter of hereditary transmission we want in fact a different mental attitude, a fuller appreciation of what heredity really is, and what it means. Heredity in the wider sense means an inherited way of life, derived from Parents and Ancestors. What a child inherits from its parents is not only the colour of its eyes, its temperament, its power of resisting disease, but its whole tendency of mental and bodily reaction to the whole of its environment, within and without.

Heredity, in fact, means the inheritance of a peculiar way of responding, not only under conditions of stress and strain, but under all the circumstances of life. There is a deep underlying truth in the Gilbert and Sullivan conclusion as to the inborn tendency to Liberalism or Conservatism in every child.

Environmental influences of course, both good and bad, help to promote or to arrest the development of these inherited tendencies and capacities. They modify, they may even radically alter the response made, but they can only do so within the limits fixed by hereditary endowment.

Now if Eugenics means, as it does mean, the application of this genetic knowledge to human life, then I have surely said enough to justify the statement that Eugenics constitutes the great problem of our time.

For if progress in art, science, and ethics, citizenship, and in the fuller life, depends largely on innate capacity of response in the individual, then it is clear that the transmission of these innate capacities to offspring through eugenic marriage becomes a question of supreme importance, and one which enters intimately into all our present day problems.

War and Peace.

Take, for instance, the question of the abolition of war and the establishment of world peace.

Both war-like and peace-loving races of mankind have existed in the past, and are still present in the world to-day; and though much can be done by education, by the removal of international misunderstandings, and by the promotion of friendly relations between nations, to promote peace—yet it is I think true to say that if resort to physical violence—as a mode of settling differences between nations, as between individuals, is to be permanently banished,—then we must set about the task of breeding and rearing peace loving citizens, that is citizens from whose natural inclinations and temperaments the wish to resort to physical violence, *i.e.,* the war-like spirit, has been eliminated, and not merely temporarily inhibited or suppressed. In other words, the only way to permanently abolish war is to change the heart, that is the *nature* of individual men and women.

Adaptability and Educability.

Or let us take the economic crisis through which our nation, like many others is passing to-day. The world troubles of to-day are mainly due to the persistence of a spirit of nationalism in a world which has largely become international in its social and economic relationships.

The quality which is especially wanted in our citizens to-day is *adaptability,* the capacity to learn by experience, the ability to appreciate and overcome difficulties of all kinds, and to overcome them by choosing the best method of re-action to them.

But adaptability and educability are inborn qualities; they vary greatly in different individuals, families, and races; they can be hereditarily transmitted from parents to children, and unfortunately they can be hereditarily lost.

You cannot produce adaptability and educability by education, although you may, by suitable training, develop these qualities up to a certain limit, if they are already present.

The successful life, that is successful in the best sense, calls for the exercise on the part of the citizen of a certain natural endowment, ability to see the other man's point of view, and to work with fellow citizens for the good of the community as a whole, in short it demands " capacity for citizenship."

It is, of course, true that these natural gifts can be improved and developed by education and training, though it is unfortunately also true that our scheme of national education has so far paid too little attention to training for citizenship, but the fact remains that the number of citizens who possess the ability to respond adequately to such training is far too small.*

We are trying to live the highly complicated life of a modern society with the mental and temperamental outlook of a more primitive age. And this applies not only to our life as a nation, but even more truly to the international life of mankind.

The only way in which we can increase the proportion of citizens who are endowed with these essential qualities is by the biological, that is the eugenic method.

The question is whether we shall simply wait, in the hope that Nature, using her old weapons of struggle and survival, will accomplish this change for us, or whether we shall be wise in time, and use such knowledge as we already possess to check the racial deterioration which there is reason to think is going on, and to establish in the future a more virile and a better endowed race of citizens.

But whichever line of action we adopt, our present attitude to this vital problem is a very inconsistent one, for while interfering with, and preventing the operation of natural selection, civilised mankind has, up to the present, failed to establish any organised system of artificial control in its place.

*See paper on "National Education from the Biological Standpoint," World's Education Conference, Denver, Colorado, 1931.

Moreover we cannot ignore the fact that, if we decide to wait and to leave this matter to natural selection, that will mean the return to the older and cruder methods of war, pestilence, and famine.

In this matter of communal life and citizenship, our mental attitude also urgently requires revision.

Not only the general public, but many educationists, and persons in authority still hold an exaggerated opinion as to the power of education to influence the lives of citizens in this forward direction. The truth is that individuals who are born without any or with very little innate capacity of response to the demands and opportunities of life in a civilised and cultured society, cannot be made into really good citizens by any amount of education and training, though they may be able to lead fairly successful lives in the simpler environment of a primitive society, or in a segregated and sheltered colony.

These and other examples must serve to emphasise the fundamental importance of the biological factors in human life, and human civilisation. Special circumstances, however, exist at the present time, in our own, and in other civilised countries, which greatly emphasise both the importance, and the urgency of the problem.

We will now consider shortly, some of the special circumstances which make for urgency at the present time.

EVIDENCE OF RACIAL DEGENERATION.

When we survey the changes which have occurred and are now taking place in the quantity and quality of our British population during the last fifty years, all thoughtful persons must, I think, be impressed with certain distrubing conclusions which force themselves on the attention.

The main facts were summarised in the Galton Memorial Lecture delivered to the Eugenics Society in

1928 and re-published in "Essays and Addresses by a Surgeon," Lewis and Co., London, 1930.

The first disquieting information was supplied by the Army Recruiting Statistics soon after the Boer War. These showed that forty per cent. of the candidates for military service had been rejected during the previous ten years owing to physical defects. Corresponding figures issued by the War office in 1929 also showed that in spite of frequent lowerings of the standard of fitness, an even larger percentage of rejections occurred in that year.

The Report of H.M. Inspector of Constabulary for the year 1924 stated that of every hundred applicants for admission to the Police Force during ten years, only five were accepted as physically and mentally fit to undertake the responsible duties of a Police Constable.

*It is interesting to note that, owing to the large number of candidates the stipulated height of the Metropolitan Police Force has now been raised from 5 ft. 9 in. to 5 ft. 10 in.

In 1908 the Act was passed which authorised the compulsory medical examination of school children.

In 1919 Sir G. Newman reported that out of some 6,000,000 children of school age, 1,000,000 were, owing to bodily or mental defects, unable to obtain full advantage from the education provided for them by the State.

It is quite true that more recent Reports show a striking improvement in the mental and bodily health, personal hygiene and general nutrition, in our school population.

The recent Report of Dr. Bashford, M.O. to the General Post Office, London, (published in the *Lancet,* December 5th, 1931), gives a comparison between a group of two hundred P.O. messenger boys, aged 16, examined as regards weight and height between the years 1905-8, and a corresponding group between the years 1929-31. The latter group showed an advance of 16 lbs. in weight, and

*_Times_, January 9th, 1932.

1½ inches in height over the corresponding sample of the previous generation. Both samples were drawn from similar London working class homes. An enquiry into similar groups of girls under the same conditions also showed a gain of 6 lbs. in weight and 1 inch in height in the latter group.

Towards this encouraging improvement, no doubt better home conditions and the earlier recognition and treatment of disease, rendered possible by the medical inspection of boys and girls during school age, in the latter groups, largely contributed.

This is all to the good, but neither in the case of the school children nor in that of the P.O. Messengers do these facts provide any real evidence of any growth in natural intelligence or innate capacity in the children and young adults examined. On the contrary, Sir G. Newman's Report for 1929 speaks of a "lack of physical stamina and power of resistance in the school population."

We fully recognise that in regard to nutrition, food, housing, and way of life generally, a change for the better has occurred in the working class population of the country, but this means that any resulting improvement is the outcome of better environmental conditions. It is a question of *Nurture,* rather than of *Nature.*

Neither these, nor other cases which might be brought forward, supply any reliable evidence that any real growth has taken place in the natural intelligence or the innate capacity for sustained work in our school or young adult population of to-day.

On the same lines we have the Report of the Government Enquiry carried out during the War, into the fitness for military service of some two and a half millions of men of military age.

This Report revealed an unsatisfactory condition as regards health and vigour in this section of the population, which it is true represented a residue after the withdrawal of the higher grade sections. It showed however that of every nine men of military age in Great Britain in 1917-18,

only three were perfectly fit and healthy in mind and body.

It is true of course, as I have already said, that a considerable proportion of this impairment of health and vigour would, and indeed did, as a matter of fact, disappear under the invigorating influences of good food, exercise, and life in the open air, and the other health promoting influences incidental to military training. The same also applies to the case of the school children.

But here again we are dealing with *Nurture* and not with *Nature*.

Let us turn then to some evidence which bears on innate capacity of the mind and on natural intelligence, namely, to the work of the Departmental Committee set up by the Board of Education and the Board of Control to enquire into the prevalence of mental deficiency in the population.

The Report of this Committee showed that there were in 1929 over 300,000 certifiable mentally defective persons, or over 8 per 1,000 of the population of England and Wales, although of this number only about 1 in 6 had been "ascertained" in the official sense by local authorities. The Report also stated that there were grounds for thinking that the number of such defectives in the population had considerably increased during the last twenty years.

Further this 300,000 does not include a larger marginal group, amounting, it is thought, to some ten per cent. of the population, of mentally sub-normal individuals.

Now in regard to this large and increasing number of mentally defective and mentally sub-normal individuals, we know that, although education and training can do much to develop such feeble capacity and intelligence as exists, they cannot eradicate the defect, for in these poorly endowed fellow citizens, as Shakespeare said of Caliban in "The Tempest,"—"Nurture will not stick." When also we recognise that these mental and temperamental

disabilities are frequently hereditary and will, in a proportion of cases be transmitted to the next generation, we begin to realise the seriousness of the position.

Let us consider for a moment:—

For over fifty years in this Country, every child has been educated, including in recent years not only the normal, but the blind, the deaf, and the defective.

For over forty years every sane man, out of prison, has had a vote, and every grown woman has one to-day.

For twenty years we have had National Health Insurance, and Health Visitors have been teaching our mothers to look after their babies; vast sums of money have been spent on the relief of poverty, and, in recent years, on unemployment. Yet, in spite of all this expenditure of time, money, and energy, what do we find to-day?

We find that 1 in every 10 of our citizens is too dull or sickly to earn a living unaided, 1 in 200 is, or has been mentally afflicted, and 1 in 120 is mentally defective.

Surely something must be wrong somewhere.

The fact is we have left Heredity out of our calculation.

We have been concerned too much with *Nurture,* and too little with *Nature.*

We have been replenishing our population from the less well endowed, and the less worthy, rather than from healthy and vigorous stocks.

The Differential Fertility Rate.

I want now to consider very shortly two important factors, which bear intimately on the problem of Racial Decay, about which we have just been speaking.

The first is known as the *Differential Fertility or Birth Rate.*

We know that the number of children or births per family increases as we descend the social scale.

For instance, the manual worker has, on the average, a larger family than the brain worker, and the unskilled than the skilled labourer.

*In the Census of 1911, the population was divided into eight social classes. In the first group, which comprised the upper and middle classes, the proportion of children born to each hundred families was 190, of whom 168 were living at the time of the Census.

In the third class, which included the skilled workers, the figures were 279 children born, and 232 surviving.

In the fifth class, which included unskilled labour, the numbers were 337 children born and 268 surviving in every hundred families. While in class Eight, which comprised agricultural labourers, the numbers were 327 born and 284 surviving.

It is probable that the 1931 Census will show a further fall in the Birth Rate in the skilled and higher paid sections of the populations. While the professional classes, both in this country and in the U.S.A., seem to be dying out, except in so far as they are being replenished by immigration from other groups.

The second factor is the *Differential Intelligence Rate.*

In regard to this problem the information is less definite, but still suggestive.

Such evidence as we possess points to the fact that children, on the average, do vary in natural intelligence according to the social status of the families from which they come.

The enquiry conducted by the Industrial Health Research Board in conjunction with the National Institute of Industrial Psychology into two groups of London Elementary School Children, though primarily undertaken from the point of view of vocational guidance in industrial occupations, did also suggest that children vary in natural intelligence according to the social level of the family to which they belong.

*Stevenson—" Fertility of Social Classes," May, 1920.

An investigation conducted by Godfrey Thompson in 1922,into a group of 3,000 Northumberland children, also points in the same direction.

More recently Dr. Evelyn Lawrence,* in an enquiry into a considerable number of children drawn from various institutions and schools has found that the intelligence quotient shifts over from above the 100 level, to below it, as we go down the social scale from the higher to the lower classes. The correlation, however, is not a high one: (it is of the order of ·2 to ·3) and this suggests that inheritance is not the only factor concerned in the case of the children whose mental qualities are under investigation.

No doubt there are difficulties inherent in the nature of this enquiry into the inheritance of natural intelligence in relation to social position. It may, for instance, be said that it is only possible to judge of mental qualities by observation of the behaviour or conduct of the individual, animal, or child, as the case may be, and inasmuch as behaviour and conduct depend on training and experience, as well as on innate mental capacities, that therefore uncertainty will be introduced which will vitiate the results of the enquiry.

No doubt the estimation of mental acquirements made is one thing while the accurate assessment of capacity to make such requirements is quite another. But the growing accuracy of the tests employed, and in the methods of using them, have enabled psychologists to make fairly accurate estimates of natural intelligence, and also to a somewhat less extent of temperamental endowment.

In regard to Temperamental Endowment, in its racial, social, and individual aspects, and to methods of testing it, I may refer to Prof. F. C. Bartlett's valuable paper on "Temperamental and Social Class," Eugenics Rev.,

*An Investigation into the Relation between Intelligence and Inheritance. *British Journal of Psychology,* Vol. XVI. See *Lancet,* January 2nd, 1932.

April 1928. And to a more recent article by Mr. P. G. Vernon, Eug. Rev., Jan. 1932, on the Development of Methods of Assessment. Both articles point to the progress that is being made in this direction.

On the other side there can be no doubt, speaking generally, that natural intelligence, like other mental qualities, is an inherited and transmissible character.

Though the question of intermarriage must not be ignored, the numerous records of families in which superior endowment in diverse directions has been transmitted through several generations, provides abundant evidence that exceptional natural gifts in music, art, science and other spheres of life show a marked familial incidence.

This being so the question remains whether social status affords any reliable indication of natural intelligence or innate capacity on the part of the parents of the children whose mental qualities are under investigation.

No doubt there are disturbing influences. Social status depends on a number of factors. There is no evidence, for instance, that musical talent or other forms of artistic ability are associated with high wage earning capacity in our social organisation of to-day. Though there is evidence that judgement, foresight, and excessive ability and temperamental qualities (Bartlett) do lead to success in life. It is certainly the case that the great thinkers, reformers and pioneers throughout the ages, from the very fact that they have been in advance of their generation, and often in opposition to the social tradition and custom of their time, cannot be included in any test which rests on social status alone.

Then, too, it is necessary to take account of the upward and downward currents which are constantly flowing through Society, currents which at times, in spite of effort carry some more worthy individual downwards, and less worthy upwards in the social stream.

Yet in spite of these and other disturbing factors, and although—as was pointed out in the Galton Lecture—

we must not assume that wage earning capacity and social status, *i.e.* "civic-worth," are entirely adequate criteria of the possession of valuable innate mental qualities or of "racial" worth, *i.e.* of capacity to hand on these good qualities to children, and so to make a valuable contribution to the human life of the next generation,—yet, it is I think true to say that, on the whole, those families whose members have shown some ability to rise in the social scale, are more likely to contribute better human material to the life of the future than families which, through lack of innate capacity, or skill, or of inferior character, have fallen to a lower social level.

But whatever may be the association between innate capacity, and wage earning power, and social status, in the general population made up as it is of citizens of average intelligence, we have still to reckon with the lowest group composed of individuals of sub-normal mentality, amounting, it is thought to some ten per cent. of the population.

In this section, which forms the so-called "social problem group," natural intelligence, social status, and wage earning capacity are absent, or present only in very small amount. But it is in this section that fertility is at its highest, and it is from this section that the nation is largely recruiting its future citizens.

Thus the evidence derived from a study of these two factors, *Differential Fertility,* and *Differential Intelligence,* tends to support the conclusion that the British nation is largely replenishing its stock to-day, not from the naturally well endowed, but from the less gifted, and less worthy sections of its population.

How has this Result come about?

But the question may be asked—how has this state of things come about?

We cannot suppose that we British citizens have deliberately embarked on a policy of racial deterioration.

What then, it may be asked, are the factors which, outside conscious control, and deliberate intention, are helping to bring this result about?

Probably the most fundamental factor depends on the fact that the action of natural selection, which, in earlier stages of human evolution, was largely responsible for the elimination of the less fit and defective individuals, has been to a considerable extent superseded in our modern civilization.

Socialised mankind, in the march of civilisation, has rightly interfered with the working of natural selection in its cruder shape, under the old order, but socialised man has not, as yet, developed any organised method of artificial control to put in its place.

As was said at the Modern Churchmen's Conference at Oxford in 1930—"While mankind has attained considerable success in acquiring control over natural forces, and over those environmental conditions which have to do with comfort, and a higher standard of life—civilised man has, as yet, made but little organised, conscious effort, to improve the quality of human life itself, or to ensure the transmission of superior mental and bodily qualities, to future generations."

The Influence of Birth Control.

A second very important factor has been the limitation of the size of the family by artificial means, that is the practice of *birth, or more properly conception control.*

It is true that, in regard to numbers,—*i.e.* the quality of human life produced from generation to generation,—the world-wide practice of conception control has, during the last fifty years, exercised a growing, and in some directions a salutary influence on the conditions of human life in civilised countries.

But as we saw, when discussing the Differential Birth Rate, the tendency of this movement of artificial limitation has been to reduce the number of births in the more highly

educated, better endowed, and more successful, rather than in the less prosperous, less educated, and less fit social groups. While in the lowest, least responsible, and mentally sub-normal section, the influence of birth control in reducing fertility has been negligible or very slight.

To this extent, therefore, the practice of conception control has exercised a dysgenic effect on the population as a whole.

Expert opinion is somewhat divided as to the relative part played by physiological, biological, and sociological factors in producing this fall in the Birth Rate, but there can be little doubt that conscious limitation of the size of the family by artificial means has in many cases, and in many countries, played an important part.

Another very important fact is that many children are being kept alive to-day, who under the old regime of uncontrolled natural selection would have been eliminated at an earlier stage of existence.

Thus Sir G. Newman mentions that in a recent year 40,000 more babies were born than in the previous year. The question is as to the innate capacity of this extra crop of human life.

Much of our social and domestic legislation to-day is not founded on sound biological principles.

We help the weak and the defective, as indeed it is our duty and privilege to do, but we aid them at the expense of the normal citizen, and what is still more serious, our present methods actually provide for the handing on of this burden of human defect, in an aggravated form, to future generations.

Eugenics and Medicine.

But any discussion of the agencies which to-day are helping, directly or indirectly, to bring about racial deterioration, must include some consideration of the influence exercised by medical theory and practice on human welfare. And at this point I propose to discuss the relation between *Genetics and Medicine*.

Under the social conditions of life to-day, in our own and other civilised countries, I fear it is true that medical science and medical practice tend to perpetuate and prolong many human lives which—owing to some inherited bodily or mental defect, or to an inborn lack of resistance to disease, would have been eliminated, and not allowed to reproduce their kind under the old order of stringent natural selection.

The great progress made in the protection of the community against infectious and contagious diseases during the last fifty years has, in the main, been due to fuller knowledge about disease conditions outside the body,—about the growth and spread of the Typhoid, Typhus, Cholera, Plague, Tetanus, and Tubercle bacillus, and many other pathogenic organisms in the earth, air, water, food, or other materials with which the human body comes into contact, and also by methods of reducing the virulence of such organisms if they do gain an entrance.

All these and many other good results have followed successful attempts to deal with the *environmental* causes of disease.

In another direction also, the so-called *"Deficiency Diseases"* have been, and are being, successfully combated by restoring to the diet of civilised communities missing accessory food factors, the absence of which is due to faulty methods of cooking, using, and preserving food.

In like manner *Nutritive Disorders* due to disturbances in the function of the endocrine or ductless glands are now being remedied by the administration of the missing hormones which these glands normally supply.

But these and other curative or remedial measures are concerned, directly or indirectly, with environmental factors, with the seed rather than with the soil.

It is not, I think, difficult to realise that the possibilities of further progress in this direction, though by no means exhausted, are still not inexhaustible.

There is, however, another field of enquiry which,

though full of promise, has up to the present been largely unexplored.

I am thinking of the human material, the soil in contradistinction to the seed, to Nature rather than to Nurture —to innate capacities of resistance—to inborn tendencies which the Physicians of an older school called "constitutional factors," in fact to inborn qualities rather than to environmental influences.

Every one, especially medical men, must be proud of the growth of medical knowledge and the increased control over disease which has taken place during the last fifty years. It is indeed true to say that medical science has undergone a new birth in our generation, and all will welcome further victories over sickness and suffering. At the same time, we are beginning to realise that, if this growing advance in medical knowledge is to secure the best results for Humanity as a whole, then the medical, like the social horizon must be extended so as to include the health and welfare of future generations, as well as our own.

Let us take one or two examples.

First then, as to the importance of Hormonic deficiency from the genetic point of view, in regard to which recent letters have appeared in the Medical Journals (B.M.J. March 7th, and April 4th, 1931).

Diabetes.

The modern treatment of Diabetes by Insulin has enabled sufferers from that disease to live a normal life and, in some cases, to marry and beget children. But we must not forget that, although continued treatment with Insulin will keep the disease in check, it will not remove the pancreatic defect on which Diabetes depends, and since this deficiency of internal pancreatic secretion constitutes an inherited defect, it will be transmitted to the children of parents so suffering, in a certain proportion of cases, especially if both parents happen to be diabetics.

The familial incidence of Diabetes is evidence of the hereditary nature of the disease. The genetical factor involved in the pancreatic hormonic deficiency which underlies this disease has been recently shown in Mjoen's observation on hybrid individuals in the cross between Norwegians and Lapps, in whom Diabetes is usually prevalent (see Eugenics Review, April 1931).

Thyroid Defect.

The same is true to a certain extent of thyroid deficiency, Myxœdema, and Cretinism.

No doubt the peculiar defect of thyroid deficiency is associated with a lack of iodine supply in the water or food, but this partial dependence on environmental and nutritional factors only carries the genetic or inborn factor a stage further back. It means that the initial trouble lies in the lack of capacity on the part of the thyroid to assimilate minute quantities of iodine.

The genetic aspect of the problem is also illustrated by the high incidence of Cretinism in certain Swiss valleys where intensive in-breeding has taken place, and also by the occasional occurrence of twins of which one may be normal and the other a Cretin, although the pre-natal environmental factors must have been similar in both twins.

From our present point of view the real trouble in Diabetes and Hypothyroidism is, that by the administration of an hormonic substance, lives are prolonged and defects perpetuated which must prejudicially affect the health and vigour of the next generation. No one would regard without anxiety the establishment of a race of individuals dependent throughout life on an artificial supply of Insulin, or Thyroxin.

Tuberculosis.

Then there is the difficult problem of *Tuberculosis*.

It seems clear from genetic evidence that susceptibility to attack by the Tubercle Bacillus differs in degree in different individuals, families, and races.

This remains true after due allowance has been made for unhygenic surroundings, mass infection, and other predisposing environmental conditions.

*With regard to the effect of race crossing on susceptibility to Tuberculosis, Mjoen's observations on two hundred Norwegians, two hundred Lapps, and two hundred hybrids between the two races, show a considerably higher incidence of Tuberculosis in the latter cross. In this connection it is significant that lung volume, as tested by the spireometer, gives markedly lower readings in the hybrids (both men and women) than in either of the pure races. The same is true of hand grasp as tested by the dynamometer.

While it is true that modern sanatorium treatment and other methods of dealing with sufferers from Tuberculosis have undoubtedly prolonged life in a considerable number of individuals who, until recent years, would have died at an earlier age, and thus presumably may have helped to perpetuate inherited susceptibility to the disease, it is also true that there has been a gradual and fairly continuous fall in the mortality from Tuberculosis in the population, which, in the opinion of some experts, is due to, either an increased immunity on the part of the people, or to a lower virulence on the part of the infecting organism. The spread of knowledge about the nature of tubercular disease and improved methods of avoiding mass infection have no doubt also contributed to this result.

However this may be in the case of the disease Tuberculosis, it will be clear from the examples already given, and others which might be mentioned, that what is wanted is not any slackening in the pursuit of medical knowledge, or of the various ways of preventing disease on

*See *Eugenics Review*, April, 1931.

the environmental or nurtural side; but the growth and spread of genetic knowledge as to the best and most practical methods of increasing natural resistance to disease in the population. We may hope that the citizens of the future will not only be endowed with the capacity to resist invasion by the many kinds of disease organisms, which now take such a heavy toll of life and health, but that they will also possess a greater power of recovery if attacked, with the added advantage of enhanced immunity to subsequent invasion by the same kind of pathogenic organism.

But we have, I fear, a long way to travel before we can secure the active co-operation of the medical profession as a whole in the eugenic movement. As I said at a discussion at the Sociological Society twenty-five years ago, and again emphasised in the Galton Memorial Lectures in 1928—" We still await the great impetus which could be given to eugenic progress by enlisting the interest and active co-operation of medical men in heredity problems. Moreover, the advantage would be mutual—on the eugenic side, because doctors engaged in general practice possess unrivalled opportunities for the collection of family records of both mental and bodily characters hereditarily transmitted, through several generations—while on the medical side the gain would be equally great, for growing medical knowledge continually emphasises the great importance of the hereditary factor in disease."

But before we can hope to secure the interest and co-operation of the Medical Profession in genetic problems, certain preliminary steps will be necessary, A more favourable attitude to the teaching of Genetics must be obtained in the General Medical Council, in the Medical Faculties of our Universities and in medical schools, in the Ministry of Health, in Local Health Authorities and Public Medical Services. The study of Genetics and Eugenics must in fact form an integral part of the theory and practice of Preventive Medicine.

There are however encouraging signs of progress in several of these directions.

We have now discussed some of the various influences which have been concerned in bringing about the racial deterioration in our population, which I described in the earlier part of this address.

Among these prejudicial agencies we have found that the most fundamental, the one which embraces many of the others, was in fact that, while modifying and largely abolishing the operation of natural selection, civilised man had not up to the present established any effective method of artificial control to take its place.

Another agency which, though possessing many counter benefits, is yet on the whole acting dysgenetically at the present time, is the growing practice of contraception. The remedy here must be not the curtailment of knowledge about Birth Control, but the spread of sound instruction, and wise advice on this most important subject to all sections of the population, poor as well as rich.

Another influence working in the same direction has been the great but indiscriminate saving of infant life, which has taken place in recent years.

Here the difficulty is not the growing interest in infant welfare and mothercraft, and the improved conditions which to day surround child life,—these are all to the good, and worthy of every encouragement; the real trouble is that the test of the fitness and worthiness, both from the civic and racial points of view, of each young life so saved has to be tried out later, when it reaches the adolescent or adult stage. This delay in knowledge involves a waste of time, money, energy, and human life on a very large scale, and the loss and the burden is borne by the State, in the shape of diminished productiveness on the part of its citizens, and an increased burden in the shape of crime, pauperism, mental deficiency, insanity, and other social evils.

The remedy here lies in a great extension of the preventive method. The formation of a " racial conscience " in the minds of the citizens, and a determination on the part of the community, as a whole, to prevent the pro-

duction of defective offspring, and the handing on of this load of human defect and misery to future generations.

The Remedies.

I have now completed this imperfect sketch of what I fear some may regard as a gloomy picture. We will turn with relief therefore to a short consideration of possible remedies, and the steps we must take if we really desire to arrest this deterioration of which I have spoken, and if we are in earnest in wishing to change human nature for the better.

These fall into two groups.

Positive Eugenics.

On the one hand we must endeavour individually and collectively, and by all legitimate means, to encourage eugenic marriage, that is marriage between individuals who are naturally well endowed, between men and women, born of healthy and vigorous stock. This constitutes Positive Eugenics.

Here it will be self-evident that any form of compulsion, with the object of forcing men and women to marry, or of choosing their partners for them must be inapplicable to human beings, however necessary such a course may be in the case of domestic animals.

Apparently one of the reasons which led Mr. Bernard Shaw to advocate equalisation of incomes was the effect such equalisation would presumably have in promoting free choice of marriage, and thereby in the production of a healthier and more virile race of human beings.*

But while social status and economic position no doubt provide opportunities and facilities for marriage, yet in civilised societies, at any rate, courtship and marriage depend on many other factors, among which mental and tempera-

*Bernard Shaw—" The Intelligent Woman's Guide to Socialism and Capitalism." See also Review of Mr. Shaw's book in the *Eugenics Review*. July, 1929.

mental endowment and natural predisposition, influence choice even more than social and economic position.

Still as regards Positive Eugenics the freer the choice in marriage the better, provided we are dealing with an educated people—that is with citizens who are fitted by nature and by training to exercise this supreme choice wisely, and with due regard to the interest of future generations.

In any case, much will have to be done by education in racial responsibility and in citizenship, before courtship and marriage are raised to a higher level, on which a sense of duty to posterity and trusteeship for the life of the future can co-operate with natural instincts and mutual attraction, in the promotion of eugenic marriage.

Here also in Positive Eugenics it will be necessary to lighten the economic burden which now presses so heavily on parenthood among the better educated, the professional, and skilled labour, social groups.

Negative Eugenics.

With *Eugenics in the Negative or Restrictive* aspect the case is quite otherwise.

Restrictive Eugenics implies the restriction of free choice of marriage, and the prevention of procreation in the case of individuals who are the subjects of hereditary defect.

Primary Amentia.

Let us take *Primary Amentia* or feeble mindedness as a type of such a transmissible defect, and consider shortly the human material with which we have to deal.

In the case of inherited mental deficiency this human material consists of (a) individuals who "exhibit" the defect in their own persons, and also carry the seeds of it in their germ cells, and (b) individuals who although they do not show the defect in their own persons, yet carry the

seeds of it in their germ cells. These we may call "carriers."

Now while all persons of the (a) or "exhibitor" group are mentally abnormal or subnormal, those belonging to the (b) or "carrier" group may be apparently normal mentally, and cannot, at present, except perhaps after careful examination of their family history and ancestry, be distinguished from normal citizens.

It is evident that these two groups will require different consideration and treatment.

In the (a) or "Exhibitor" group, attempts to prevent procreation by appeals to religion, or morality, or to citizenship and public duty, will fall on deaf ears, owing to the lack of normal intelligence and self control in such defective individuals.

In dealing with this group we are left with three possibilities—Segregation, Socialisation, and Sterilisation, or with the combined use of any of these methods.

Segregation.

Complete segregation of all "primary aments," if carried out for the reproductive period, which in the male means practically the whole life, if rigidly carried out, would no doubt prevent procreation in this "Exhibitor" group. Segregation is, however, a costly method, and at present is not being carried out on any adequate scale.

Thus Miss S. Lawrence stated in reply to a question in the House of Commons on March 12th, 1930, that only nineteen out of nearly two thousand Local Authorities had then provided any institutional provision for mental defectives.

Even if segregation were carried out, it would not deal with "carriers," of the defect and it is from "carrier" rather than from "exhibitor" parents, that by far the larger number of mental defectives arise.

Socialisation.

This means returning a certain proportion of higher grade defectives, who are free from anti-social tendencies, to communal life, under adequate supervision or guardianship, and after they have undergone a period of education and training during institutional life.

This method is less costly than segregation, and may be successful in cases where supervision and control are adequate.

The risk that, in spite of such oversight, procreation may occur is however considerable in many cases.

Indeed parents and guardians, ignorant of the biological factors involved, are apt to be unduly influenced by the general improvement in health and conduct which many higher grade defectives show, when placed under favourable conditions, and they may think that such individuals are fit to be entrusted with still more freedom, that is with marriage and parentage.

But they forget that the germ cells will still remain uninfluenced, and if fertilised, will develop into offspring after their kind, and that a large proportion of such offspring will themselves be defective.

Sterilisation.

There remains then the question of Sterilisation, not as an alternative, but as complementary to the preceding methods.

I cannot now describe in detail the reasons which have led the Eugenics Society in London and many influential citizens to advocate sterilisation of the voluntary kind, carried out in suitable cases, and with adequate safeguards, as a method of preventing the transmission to offspring of serious mental and bodily defects of a transmissible kind.

Sterilisation is, and should be regarded as one method of conception control. The ethical aspect of sterilisation for eugenic reasons has been dealt with by many writers, while in regard to the biological aspect of the subject of contraception, I may perhaps refer my hearers to my article in the *Encyclopædia Britannica* (last Edition.)

I can only express the opinion, and experience supports it, that, as biological and genetic knowledge grows and spreads, as a fuller realisation of the duties of citizenship increases, and a racial conscience is aroused in the minds of the citizens, voluntary sterilisation will be looked upon as a privilege and not as a deprivation, and as a way out of a great difficulty by many married persons, and as a means by which conflicting individual and racial interests may be harmonised.

In conclusion, may I say that in thus emphasising the importance of the biological factors in human development and human life, I do not wish in any way to underrate the important part played by environmental influence.

We owe our way of life, our social heritage, to our ancestors, just as we inherit from them our inborn powers of mind and body, though in quite a different way.

A great step will, however, be gained if we are led to realise more deeply, and to act on the conviction, that the future of our race and nation, and it may be the future of our Western Civilisation, depends in the last resort, not on Governments or on Acts of Parliament, but on the capacity, the mental and bodily qualities, in fact the biological attributes of our citizens.

But innate capacity in its many spheres, and in all departments of life is a matter of breeding. It is in fact a eugenic problem.

May we not hope then, that just as our rude awakening to the gravity of the economic position may have only

come just in time to avert economic disaster on a world-wide scale—so too that our tardier realisation of the far greater peril of racial decay may not be delayed until it is too late?

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ON CERTAIN ASPECTS OF HUMAN BIOLOGY

BY

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PREFACE

LECTURES IV and V in this Course, describing the results of an inquiry into certain aspects of the physiology of the Red Blood-cell, are here published separately.

The titles of the remaining three Lectures, which will be published later, are—

LECTURE I: On the Genetic Significance of Hemilateral Asymmetry in the Vertebrate Organism.

LECTURE II: On the Making of Use-Acquirements, as illustrated by the Neuro-Psychic and other Responses.

LECTURE III: Genetics and the Preventive Medicine of the Future.

LECTURE IV

ON THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE SHAPE AND CONSTITUTION OF THE RED BLOOD-CELL

In a letter to 'Nature' some years ago, Eric Ponder* described certain observed "changes in the shape of mammalian red cells as due to the presence of a cover-glass," and he associated the assumption by the cell of the spheroidal form, with proximity to two closely opposed glass surfaces, when a drop of a suspension of red cells in N.S. solution is examined on a slide.

The following observations, carried out during the last four years, were undertaken in order to analyze more closely the factors concerned in these changes in shape of the red blood-cells, when suspended in N.S. fluid.

Human red blood-cells, washed and suspended in normal saline, were first examined in hanging-drop preparations, and it was found that while the cells in the centre of the drop preserve the biconcave disc shape, those at the periphery, where the layer of fluid thins out, become crenated or "prickled," while a few spheroidal cells of smaller diameter will also be seen. In the hanging drop the possible influence of a cover-glass has been eliminated.

If the cells in the suspended drop be agitated with a grease-free glass rod, the majority will assume the prickled condition. If, however, the rod be first greased, or coated with solid paraffin, then the change in shape is prevented or only occurs more slowly.

* 'Nature,' November 10th, 1928.

If the glass slide from which the inverted drop hangs is first coated with a film of fat, the cells retain the discoid shape.

Repeated washing in normal saline (three or four times) or, as we shall see later, washing in a hypertonic saline fluid, causes most of the cells to become prickled.

Effect of Fat Solvents on the Red Cell.

If to a standardized suspension (1 drop of blood to 2 c.c. of normal saline) of once washed red cells, we add a drop of a 4% solution of pyridine in N.S. and examine the mixed suspension on a slide, without a cover-glass, the cells will shrink slightly in diameter, and will show the prickled condition, while a few cells will become spheroidal in shape.

Thus the fat solvent, pyridine, serves to bring about more quickly the change in shape which agitation with a foreign body, or repeated washing in N.S., produces more gradually.

It will be convenient to regard this pyridine-treated drop of washed red cells in N.S. as the standardized preparation of prickled cells, or shortly the "P.P.R.C. preparation" for testing the deprickling capacity of various substances.

The presence of a very small amount of blood-serum prevents this change in shape, and if the amount be sufficient, will restore the prickled cells to the normal disc form, as was pointed out by Ponder.

The Deprickling Effect of Soap (Sod. Oleate) Solution.

The next step forward arose out of an observation made by Mr. F. Young, that the addition, to a standardized suspension of red cells, prickled by pyridine, of an equivalent volume of a dilute solution of sodium oleate (at pH 7 or 7·5) in normal saline, restores the prickled cells

to the discoidal shape, just as the addition of a small amount of blood-serum does.

The strength of the soap solution used is important; a too weak solution, *i. e.* below ·01%, fails to deprickle the cells, while a too strong solution hæmolyses them. A solution of ·02% is generally useful, while a solution of ·005% under certain conditions of neutrality, temperature, etc., brings about a condition of unstable equilibrium, in which the cells pass quite quickly from the discoidal to the prickled form, and back again in the reverse direction.

This rapidly alternating change of form seems to depend on temperature, and on convection currents, in the medium, and can be set going by gently breathing on the exposed drop of red-cell suspension.

The alternating passage from the disc to the prickled state, and *vice versâ*, can also be produced by adding to a suspension of washed red cells in a test-tube a sufficient volume of 4% pyridine solution in N.S. to induce prickling.

The red cells so treated are then washed two or three times in N.S., to remove the excess of pyridine. When a drop of the suspension is examined on a slide, the cells will show this alternating passage from the prickled to the disc form and *vice versâ*.

The pH Value of the Soap Solution Used.

I have already stated that, in addition to the strength, the pH value of the soap solution is important. In the first place, if the solution is too strongly alkaline (above pH 8), this will prevent the prickled cells from resuming the disc shape. Further, oleate of soda solution ·01 or ·02% is apt to lose its deprickling capacity on standing, through precipitation of the soap out of solution, in a slightly acid medium. The re-solution of the soap and the restoration of frothing capacity on shaking, and of deprickling capacity, can be restored by making the

solution slightly alkaline (pH 7·5), or better still, by the use of a freshly prepared neutral soap solution of ·02%.

The fact that soap is a *surface-tension reducing substance* suggests that the presence of similar surface-tension reducing substances in the blood-plasma might be responsible, with the plasma proteins, not only for causing prickled red cells to resume the disc form as in our experiment *in vitro*, but also for keeping the cells in a stable discoidal shape in the blood-stream.

An attempt was therefore made to recover any such surface-tension reducing substances from the blood-serum, capable of deprickling prickled red-cells.

Some 8 oz. of clear sterile blood-serum obtained by aspiration from the pleural cavity having a drop number of 63 as tested by the stalagmometer, and capable of rapidly deprickling prickled red cells, was treated with isotonic Ca chloride solution, and a small quantity of NaOH (1% solution) was added to make the mixture alkaline. The turbidity so produced gradually settled down (on standing for twenty-four hours) as a sticky precipitate of calcium phosphate, with possibly an absorbed calcium stearate, or oleate moiety derived from the serum.

The precipitate was collected after centrifugalization ; it was washed twice in normal saline, and was then treated with chloroform after acidulation with tartaric acid.

After standing, one or two drops of the chloroform layer containing the fat were allowed to evaporate on a slide. The film of fat so deposited was washed in N.S. solution. It consisted of oil-like droplets, fluid at room temperature.

A drop of a standard suspension of prickled red cells was then deposited on the film so prepared.

The cells assumed the biconcave disc shape. The preliminary treatment with Ca chlor. and NaOH may, however, be omitted. Thus blood-serum of known deprickling capacity in the fresh state is shaken up with an equal volume of chloroform, and the mixture is allowed

to stand some hours. Two or three drops of the emulsified chloroform layer are pipetted off, and evaporated to dryness on a slide. A fat film is deposited, which is capable of deprickling a standardized suspension of prickled red cells.

The following experiment throws light on the nature of the extracted deprickling substance.

A freshly made ·02% solution of oleate of soda is shaken up with an equal volume of chloroform, and allowed to stand some hours.

The mixture separates into three layers.

A water layer above, a chloroform layer below, and a slightly turbid intermediate layer of soap deposit at the chloroform-water interface.

Two drops from each layer are evaporated on separate slides, and tested later with the red-cell suspension. The fluid from the water layer has *no* deprickling effect, that from the chloroform layer a *slight* effect, and that from the intermediate soap layer a *marked* deprickling effect on the red cells.

The surface-tension reducing capacity of the soap solution can be tested by adding pyridine to the solution, thus :

The drop number of water is . . . 57

In comparison with water—

The drop number of oleate of sod. 1% . 153

,, ,, ,, ,, ·01% . 86

,, ,, ,, ,, ·001% . 58

The drop number of a soap solution of known strength can be raised by adding pyridine, and again lowered by reducing the concentration of pyridine in the solution.

The pyridine also raises the pH value of the solution on the hydrogen ion or alkaline side.

Thus the action of pyridine on soap solution is to reduce its surface-tension reducing capacity, and at the same time its deprickling effect on red cells.

If pyridine be added to blood-serum the effect is much the same.

Thus the drop number of pooled samples of human blood-serum was found to be *63*.

Pyridine, 4% N.S. solution, increased this number to 89. The effect on the pH value of the serum was slight —a rise from 7·5 to 8 only.

Thus the fat deposited on the slide probably acts as a surface-tension reducing substance on the coats of the red cells.

The Deprickling Factor in Blood-Serum.

A routine examination of a considerable number of human blood-serums soon showed a considerable difference in deprickling capacity in different sera.

No constant difference in this respect has been noticed between sera giving a *positive* and those giving a *negative* Wassermann reaction.

Lipæmic blood-sera often show a low deprickling capacity. After standing in a test-tube for some hours, such a turbid serum becomes clear by the rising of the fat-globules to the surface as a "fatty cream" layer. A drop of the clarified serum below this fat layer will often show an increased deprickling capacity.

The Soap Content of the Blood-Serum.

We have already seen that the concentration of sodium oleate in N.S. solution necessary to change prickled red cells in N.S. suspension back to the disc form is about ·01 to ·02%, and the pH value of the solution should be from 7 to 7·5.

C. P. Stewart and A. C. White* have shown that the amount of soap or free fatty acid (calculated as oleic acid) in normal blood, is for the blood-serum 18 to 40 mgm. per 100 c.c. for plasma 46·7 to 98·7 mgm. per 100 c.c., or roughly 1% of soap.

* See 'Biochemical Journal,' 1929, xxiii, No. 6.

We have seen that a concentration of ·01 to ·02% of soap (ol. of sod.) solution in N.S. is capable of deprickling washed prickled red-cells suspended in N.S.

Thus the amount of soap in the blood-plasma (apart from serum proteins) would seem to be five to ten times stronger than is necessary to preserve the red cells in the normal disc form.

The Part Played by the Serum Proteins in Preserving the Disc Form of the Red Cells.

Serum Proteins.

We must now consider what part the protein constituents of the blood-serum play in stabilizing the disc form of the red cells.

These proteins may be considered under three heads :

Euglobulin.
Pseudoglobulin.
Serum albumin.

The Englobulin.

A measured volume of blood-serum, which in the fresh state deprickles (+++) red cells, is treated with distilled water and normal acetic acid 6% solution, according to the Hartley method.

The euglobulin constituents are precipitated. This precipitate is (after centrifugalization) washed in distilled water and redissolved in normal saline, and brought to a pH value of 7 or 7·5.

A drop of this euglobulin solution is then tested with a drop of the standardized suspension of pyridine prickled red cells. The deprickling effect will be absent, or very slight in amount, while the agglutinating effect on the red cells will be marked, if the original serum from which the euglobulin has been obtained possesses the agglutinin necessary to clump the red cells used.

From this experiment we learn two important facts :

1. That the deprickling factor is not thrown down with the euglobulin precipitate, and—

2. That the specific agglutinating factor *is* carried down with the euglobulin precipitate, and can be recovered when this is redissolved in weak saline.

The Serum Albumin.

The clear liquid from which the euglobulin constituent has been precipitated is next dialysed against water, and the fluid from which the acid and salts have been removed by dialysis is brought to a pH value of 7 or 7·5. This fluid contains the serum albumin, as shown by the abundant precipitate thrown down by boiling after re-acidulation with acetic acid.

This solution of serum albumin, from which the euglobulin element has been removed, is then tested in the same way with a drop of the suspension of prickled red cells.

It will be found to have a marked and rapid deprickling effect. The cells will resume the biconcave disc shape, but there will be no clumping of the cells.

This experiment shows that, unlike the euglobulin, the serum albumin has little or no agglutinating, although a *marked deprickling effect* on the red cells.

Pseudo-globulin.

I have not been able to test freshly prepared pseudo-globulin, but a sample of the dried substance prepared from horse-serum, kindly given me by Dr. Hartley, when redissolved and neutralized failed to give the deprickling effect when tested with prickled red cells. Further, no agglutinating effect on the washed cells was observed.

With regard to dried specimens of euglobulin, it is also interesting to note—as has been pointed out by Dr. Hartley—that this substance under such conditions becomes " denatured " on keeping, and it is probably

this change, due to age, which accounts for the failure to obtain any deprickling effect with the dried preparation.

A sample of recrystallized horse-serum albumin, on the other hand, redissolved in N.S. and corrected for pH value, gave a marked deprickling effect on prickled red cells.

These observations on the dried crystallized substances confirm those already made on the wet, freshly prepared protein constituents of the blood-serum, as regards deprickling capacity.

The Part Played by Serum Lipoids and Soaps.

In order to study the effect on the red cells of the lipoids in the blood-serum, two samples of human sera were shaken up and allowed to stand, each with an equal volume of chloroform for some hours :

> Serum (*a*) (21), a clear serum, which in the fresh state gave a plus deprickling (D+++) and a plus agglutinating effect (A+++) on a standard suspension of my own washed red cells.
>
> Serum (*b*) (3), which gave a D— and an A— effect with the same red cells.

Two drops of the water (serum) emulsion layer from the deprickling and agglutinating serum (*a*) give a well-marked D++ and A++ effect, while the corresponding water emulsion from the non-deprickling, non-agglutinating serum (*b*) gives no deprickling or agglutinating effect on the red cells.

Two drops of the lower chloroform layer, after evaporation to complete dryness on a slide, give a fat film which, when treated with a drop of the standard R.C. suspension, gives a deprickling but no agglutinating effect in the case of both sera (*a*) and (*b*).

This experiment shows that, while the lipoid constituent extracted by chloroform from both sera deprickles red cells, the agglutinating substance is not extracted by

chloroform, but remains in the water layer, in association with the euglobulin constituent.

It may be that the seeming discrepancy in which a deprickling substance has apparently been extracted by chloroform from a blood-serum which, in the fresh state, does not deprickle red cells, can be explained by supposing that the red cells in the case of both sera are prevented, by the intervention of a lipoid film, from coming into intimate contact with the " water wetable " glass surface of the slide.

If not, then we may perhaps suppose that the serum protein and lipoid are linked together in the non-deprickling serum (*b*), and that the chloroform has dissociated the two substances and set free the lipoid element, while in the case of the deprickling serum (*a*), the free lipoid is present in sufficient amount to deprickle the red cells. In this connection we may recall the suggestion (see Cole, ' Practical Physiological Chemistry,' 6th edition, p. 46) that such a combination between protein and lipoid is responsible for the non-solubility of euglobulin in distilled water.

Taking all the facts into consideration, the latter would seem to be the more probable explanation.

A word may be added here concerning the different layers after treatment with chloroform.

The bottom or chloroform layer, *c* (in the case of a B.S. which gives a turbid layer, *c*, with chloroform), consists of chloroform globules in an aqueous (serum) continuum in which the globules are small and numerous, and well stabilized by a coating of some emulsifying substance, which, in the case of B.S., is probably not soap, since strong HCl does not break up the emulsion, but is, therefore, probably proteid.

The same bottom, or *c* layer, in the case of a B.S. which gives a nearly clear layer with chloroform consists of much larger and less numerous chloroform globules in an aqueous (serum) continuum, in which the globules are only slightly coated, and the emulsion is very unstable and readily breaks up.

The Relative Importance of Lipoids and Proteins as Deprickling Agents.

But if we assume, as indeed we must, that serum lipoids and proteins are *both* concerned in the deprickling process, the question still arises as to the relative importance of each in preserving the normal flaccidity, size, and disc form of the red cells in the blood-plasma.

The following experiment bears on this point: A known volume of a deprickling serum (Serum 21) was extracted five times with an equal volume of chloroform, and the deprickling capacity of the fat film from the first chloroform extract compared with that of the fifth extract.

The latter gave a very slight deprickling effect of the reversible kind.

The agglutination effect was absent in both films.

On the other hand, the water extract, which contains the serum proteins, both in the wet condition and after evaporation to dryness and re-solution in water, shows no diminution in either deprickling or agglutinating capacity.

This shows that the serum protein, *i. e.* serum albumin, in the absence of fats, still exercises a stabilizing or deprickling effect on washed red cells, which tends to keep the cells in the normal discoid shape.

In the course of this experiment the interesting fact was observed, that after evaporation to dryness and re-solution, the blood-serum (21) loses its deprickling but not its agglutinating capacity.

The explanation of this fact is not immediately evident, but may depend on oxidative changes.

This point will be referred to in the next lecture.

Certain Other Constituents of the Blood-Serum.

As regards certain other constituents of the blood-serum (ox and horse) included in Hartley's table*—cystine, histidine, lysine, arginine—it is of interest to find that a

* See 'Biochemical Journal,' viii, No. 5.

solution of histidine, approximately 1% in N.S. corrected to a pH value of 7, deprickles red cells D +.

Histamine hydrochloride, treated in the same way, gives D ++.

A 1% solution of arginine in N.S. made up to pH 7 at first contracts prickled red cells into spheroidal cells; later, on standing twenty-four hours in a moist Petri dish, some of the cells become disc-shaped, some helmet-shaped, while later some crystallization of the hæmoglobin occurs in the form of intra-corpuscular plates or needles.

Some Other Conditions which Influence the Change from the Disc to the Prickled and the Globular Form.

Washed red cells are extraordinarily sensitive to environmental conditions, and we must now consider some of the many factors which are concerned in the change of shape undergone by red cells under such altered conditions.

pH Value.

Among these we may first consider the pH value of the medium.

Two tubes are taken of normal saline, in one of which the solution (*a*) has been made slightly acid (pH 6) by the addition of ac. sod. phos. solution in N.S., and the other, (*b*), slightly alkaline (pH 8) by the addition of alk. sod. phos. solution in N.S.

The red cells in a standard suspension of washed cells will retain the disc form when a drop of the acidified saline solution is added, while the cells treated with the alkaline solution will assume the prickled or the globular form.

Further cells so prickled by immersion in the alkaline medium can be restored to the disc form by re-acidification to pH 6 by the acid solution.

The effect of an acid saline solution, in increasing degrees

of acidity, is to convert disc-shaped cells into helmet-shaped, and then into globular cells, ending in hæmolysis, while the effect of the alkaline solution under the same conditions is to convert disc-shaped into prickled, then into globular cells, finally ending in hæmolysis.

Moreover, besides change of contour, important changes in the diameter of the cell occur.

The biconcave disc, or biscuit-shaped cell, has the largest diameter, normally about 7·6 to 7·9 microns.

The prickled cell has a smaller diameter, and is harder and more rigid than the disc-shaped cell.

The globular cell has generally a still smaller diameter, and represents the penultimate phase just before hæmolysis.

Effect of Temperature.

If two drops (on a slide) of a standard suspension of once-washed red cells in N.S. be placed in an incubator at 37° C. in a moist Petri dish, all the cells will become prickled after three minutes. If the slide be then exposed to a low temperature, just above freezing, the prickled cells will rapidly revert to the disc form.

The presence of a small quantity of serum will prevent or delay the prickling process.

This alternate prickling and deprickling by heat and cold can be repeated on the same drop of red cells several times, until the cells "get tired" and fail to re-assume the disc shape when transferred to cold surroundings. An exposure of a quarter of an hour to the incubator temperature injures the cells, and prevents them from re-assuming the disc shape when cooled. Human (and probably all mammalian) red cells, when suspended in a saline fluid freed from blood-serum, are extremely sensitive to slight changes in temperature. Prof. A. Fleming has drawn attention to a difficulty which may arise when carrying out blood-group testing at room temperature, a difficulty which is due to the occurrence, in certain cases,

of auto-agglutination (see 'Brit. Med. Journ.,' October 31st, 1931).

This matter will be dealt with in the next lecture.

In order to obtain and keep washed red cells in the disc form for more than a few minutes, the temperature of the saline fluid in which the cells are suspended must be kept below 60° F., or the fluid must contain a sufficient amount of blood-serum or other surface-tension reducing substance to keep the cells in the disc form.

This is a point of considerable importance in the summer months, when the room temperature may be considerably above 60°.

If a drop of a suspension of prickled cells be allowed to dry gradually on a slide, the cells will revert to the flattened disc form as the drying takes place.

The Effect of Hypo- and Hypertonic Solutions.

In view of the foregoing observations on the influence exerted by (*a*) albuminous and (*b*) soap-like substances on the shape of red cells, it is desirable to review the action of hypo- and hypertonic saline solutions on the red cells.

We know, of course, that when a 2% sod. chloride solution is added to an equal volume of a suspension of washed red cells in N.S., the cells prickle or crenate and become smaller in diameter.

With a hypotonic $\frac{1}{2}$% saline solution the red cells retain the flattened disc shape, they become more flaccid, and if the strength of the sod. chloride is further reduced they undergo hæmolysis.

In an isosmotic saline fluid the cells retain the disc shape for a considerable time.

Cells which have become prickled or globular, as the result of immersion in a hypertonic saline fluid (provided the temperature is below 60° F. and the pH is 7), can be restored to the disc shape by washing in normal saline, though this re-conversion can only be successfully done once or twice.

The addition of a ·02% soap solution or a drop of blood-serum will produce the change.

Whatever may be the way in which the hypertonic solution produces the crenated change in shape of the red cell, which is usually regarded as due to osmosis, the fact that it is reversible shows that no irretrievable damage is done to either the envelope or contents (stroma) of the cell.

The Effect of other Saline Solutions on the Shape of the Red Cell.

I have already mentioned that repeated washing of red cells in normal saline (pH 7) will cause many of the cells to become prickled.

This effect may be due to the intimate contact between the cells brought about by centrifugalization during washing, whereby the integrity of the lipo-protein envelope is interfered with, or may be partially rubbed off the cell.

Samples of red cells from the same individual have been washed under similar conditions as to temperature and pH value in isosmotic solutions of—

Sod. chloride.	Magnesium chloride.
Pot. ,,	Ca chloride.
Lithium chloride.	Ba ,,
Strontium ,,	

The first, or sodium, group tends to keep the cells pliable in the disc form.

The second group (the earthy bases) tends to harden, shrink and prickle the cells, and this result is specially marked in the case of Ba chloride.

If a drop of a suspension of red cells, twice washed in Ba chloride isosmotic solution, be examined in that solution on a slide, the cells will be found prickled and later spheroidal in shape.

Such cells require a larger dose of soap solution to

restore them to the disc shape than cells washed in N.S. and prickled with pyridine.

On the other hand, cells washed in an isosmotic solution of sod. sulphate, and similarly examined in that solution, retain the flattened biconcave disc shape, and such cells require about three times the amount of pyridine to prickle them than cells washed in an N.S. solution.

Ba chloride solution hardens, prickles and contracts the cell and delays hæmolysis.

Sod. sulphate solution softens and flattens the cells and favours hæmolysis.

Effect of Filtration through Porcelain.

It has been shown (see articles in the 'Brit. Med. Journ.,' December 18th and 25th, 1920, on autoagglutination)* that the hæmagglutinating substance is removed from an agglutinating serum by filtration through a porcelain candle, under reduced atmospheric pressure.

The same filtration also greatly diminishes the deprickling capacity of a serum, any slight remaining deprickling effect disappears on standing, and on re-agitation the cells revert to the prickled state.

The filtered serum only froths slightly when bubbled with air, while the same unfiltered serum froths strongly and the bubbles are more persistent.

Thus the effect of the filtration is to remove the surface-tension reducing substances present in the blood-serum.

The Effect of CO_2 on Red Cells.

If a drop of the standard suspension in N.S. of washed red cells be mixed with an equal volume of carbonized normal saline solution (that is, N.S. which has been

* In the above articles in the 'Brit. Med. Journ.,' I drew attention to the fact that the size of the agglutinin molecular aggregate seems to correspond to the size of the pores of a filter-candle of moderately fine texture. It may be possible by the use of Dr. W. J. Elford's graded collodion membranes (see 'Rep. Med. Res. Council,' 1930, p. 31) to determine the actual size of the different agglutinin aggregates.

carbonized by the addition of solid frozen CO_2), the red cells become prickled.

The same effect can be produced more slowly by bubbling CO_2 gas, or expired alveolar air, through a suspension of washed red cells in N.S. solution.

Now the effect of carbonizing the N.S. solution or of bubbling the weakly acid CO_2 gas through a suspension of washed red cells is to shift the pH value from neutrality (pH 7 or pH 7·4) to the acid side (pH 6).

We know, from previous experiment, that the result of treating washed red cells with a weakly acid saline, for instance ac. sod. phos. pH 6, is to keep the cells in the disc form, and to deprickle any prickled cells.

But CO_2, although it acts as a weak acid, and lowers the pH value of the saline to pH 6, behaves as an alkali as far as its effect on the shape of the red cells is concerned, it *reduces them in diameter, stiffens and prickles them.*

Further, red cells which have been contracted and prickled by treatment with CO_2 can be again softened and restored to the flattened disc form, by treatment with ac. sod. phos. pH 6, or by oxygenated saline, *i. e.* normal saline through which oxygen gas has been bubbled.

This is an important fact, because, although the experiment has been concerned so far with washed red cells only, it may throw some light on the alternating phases of gaining and losing oxygen, which forms the essential element in red-cell metabolism.

It is therefore of interest to know that in the case of washed red cells the effect of exposure to carbonic acid (*i. e.* carbonization) on the shape, rigidity and diameter of the red cell is the opposite of that produced by exposure to oxygen.

The Effect of CO_2 on Soap Solutions and Blood-Serum.

In view of the deprickling effect of a weak soap solution on red cells, it is of interest to find that, as we should

expect, bubbling CO_2 gas through a sod. oleate solution of ·01% lowers both the drop number from 72 to 62, and the pH value from pH 7 to pH 6, and at the same time slightly diminishes its deprickling capacity.

On blood-serum, on the other hand, aëration with CO_2 gas has little effect on either drop number or pH value.

The Effect of Oxygen on the Shape of Washed Red Cells.

It is of interest to compare the effect of CO_2 with that of oxygen on the shape of washed red cells.

Oxygen gas bubbled through N.S. solution does not alter the pH value of the solution, which should be pH 7. When such an oxygenated saline solution is added in equal volume to a suspension of washed prickled red cells, the effect is, rather slowly, to deprickle the cells, and to cause them to revert to the disc form. The effect is thus opposite to that produced by a CO_2 saline solution.

The effect of bubbling oxygen gas through blood-serum is to slightly reduce its pH value and its drop number. The deprickling and agglutinative (if present) capacity are not materially affected.

Soap solution ·02% in N.S. solution after aëration with oxygen gas increases in hæmolytic activity.

Many of the disc cells assume the helmet shape, some are flattened with a bilateral biconvexity resembling a biconvex lens set in a rim of flat glass, or the central swelling may be unilateral, and there is a marked tendency at a later stage for the cells to stand up on their rims.

The Effect of De-Oxygenation.

If a drop of a suspension in N.S. solution of once-washed (disc-shaped) red cells on a slide be placed in a Petri dish in which some pyrogallic acid solution, to which a

little NaOH has been added, is also placed in a watch-glass, the disc-shaped cells will rapidly become prickled.

They can be deprickled by removing the slide and adding to the suspension a little oxygenated N.S. solution, but not by N.S. solution alone.

The prickling and deprickling process can be repeated on the same red cells several rimes, while the cells in a control suspension placed in a damp Petri dish *without pyrogallic acid* remain disc shaped.

The Effect of Ozone and Ozonized N.S. Solution on Red Cells.

A word or two may be added about the effect of exposing washed red cells to ozone.

If a hanging-drop suspension, in N.S. solution, of prickled red cells be inverted over a stream of ozone rising from an ozone producer, the effect will be to restore the disc shape of the cells and in a short time to hæmolyse them. The presence of a small amount of blood-serum delays the hæmolytic change.

If ozonized N.S. solution be added to another drop of suspended washed cells the effect is the same, but more rapid, but this is probably due to reduction of the pH value to pH 6.

The effect of exposing a soap solution or a dried soap film to ozone is to increase its deprickling capacity.

With blood-serum so exposed the opposite effect is produced. The serum loses its deprickling capacity, but retains its agglutinating capacity (if such is already present).

With cerebro-spinal fluid, both normal fluid and fluid with a raised protein content, the result is different. Ozonization does not markedly diminish either the deprickling or the agglutinating capacity.

In general it may be said that in regard to its effect on the form of the red cells ozone acts like oxygen, but in a more powerful manner.

Effect of Contact with Living Leucocytes and Endothelial Cells on the Shape of Red Cells.

When speaking of the deprickling effect of the fat film deposited from an evaporated chloroform extract of blood-serum on a slide, the suggestion was put forward that the effect on the red cells was due to the fact that the fat prevented intimate contact between the red cells and the glass slide.

The following observations bear on this point:

A film of living emigrated leucocytes on a slide is first obtained, by incubating two or three drops of blood in a glass (Ponder's) cell. The clot is removed, and the leucocyte film washed free from serum in a gentle stream of N.S. solution. A drop of a standard suspension of prickled red cells is then deposited on the leucocyte film so produced.

After standing a few minutes, most of the red cells in contact with the leucocyte cells will have resumed the disc shape, whereas any isolated red cells in contact with the glass, outside the film, will retain the prickled form.

If, however, the emigrated leucocytes forming the film have been previously killed by drying, then contact between it and the red cells has no deprickling effect.

If, instead of emigrated leucocytes, an endothelial cell film is prepared, by incubating two or three drops of pleural fluid containing endothelial cells (or, better still, the serous synovial fluid from an inflamed joint), and washing the film in normal saline in the same way, then the deprickling effect on the prickled red cells will be even more marked than in the case of the leucocyte film.

This experiment can be repeated several times with the same film, by washing away the red cells, and by substituting a fresh drop of the standardized suspension.

Washed pus-cells, if living, and not too much devitalized by age, or by bacterial toxins, can be incubated to form a film of adherent active, living cells, and such a film,

after washing, will give a marked deprickling effect on prickled red cells.

The fact that a film of dried, dead cells has little or no deprickling effect is important, and suggests that as long as the cell is alive, the secretion of a lipoid or soap-like, or possibly albuminous, substance on its surface goes on.

An attempt was made to make a water or saline extract of this soapy material by allowing the cells to stand in N.S. for some hours. This watery extract exercises a slight deprickling effect on prickled red cells, which is, however, reversible in character. A chloroform extract of the same cells deposited on a slide, after evaporation of the chloroform, deprickles red cells more actively.

Now it is, of course, well known that while circulating in the blood-stream under normal conditions, the red cells preserve the biconcave, biscuit, or disc shape. They do not adhere either to each other, or to the leucocytes, or the cells which form the lining of the capillary tubes, through which the red cells circulate.

The observations now recorded show, I think, that the red cells are doubly protected against danger of injury to their lipo-protein envelopes by friction or contact with neighbouring cells :

They are protected first by the surface-tension reducing substances, lipoids, soaps and proteins present in the blood plasma ; the relative importance of the protecting influence exercised by these different groups of substances requires further investigation ; and secondly, by the surface-tension reducing substances (probably lipiodal in character) which are formed by the endothelial and other cells, which form the lining of the capillary tubes.

By such arrangements the red cells are prevented from coming into too intimate contact with foreign tissue-cells, which might present an injurious foreign body surface contact.

We know, for instance, that when, from any cause such as chemical or mechanical injury, or through the

action of bacterial toxins, these living endothelial cells have been injured, then there is a tendency to a delayed current, to stasis of red and white cells, and to clotting of the blood-plasma in such injured areas. It is a significant fact that the red cells in such thrombosed sites and in extravasated blood do not preserve the disc form.

Lord Lister's classical experiment with "the living test-tube" showed the effect of contact with a living cell-wall in preventing the coagulation of the blood-plasma.

The observations herein described are concerned with a similar influence on the red blood-cells.

Here, too, we find the necessity for avoiding a too intimate contact between these cells and a water-wetable, dead, or foreign surface.

The fact is that the red blood-cell is, of all animal cells, the most sensitive to environmental influences like temperature, oxygen supply, pH value, etc. Moreover, the fluid medium in which the cells circulate is correspondingly wonderfully constant in composition.

Summary.

We have now described some of the more important influences which bring about these striking alterations in the size and shape of washed red cells, and we must now quite shortly sum up the facts and conclusions at which we have arrived.

I wish to make it clear at the outset that the changes just described have been observed so far only in cells *after* withdrawal from the blood-stream, and when removed from their normal, or blood-plasma, environment.

Cells examined in plasma or serum immediately after withdrawal from the circulation, although in certain pathological states they may show slight departures from the regular biconcave disc shape, do not as a general rule become either prickled in outline or spheroidal in shape.

I am hopeful, however, that, as more delicate means

are perfected of observing such abnormalities, it will be possible to demonstrate some change in size or shape, even in red cells while circulating in the capillaries.

Among the more important facts noted, the following may be mentioned :

The extreme sensitivity of the red cell in a standardized suspension to slight changes in the pH value of the solution employed.

Thus solutions on the *alkaline* side of pH 7 (*i. e.* above pH 5) tend to contract, stiffen and prickle washed red cells, while solutions on the *acid* side (*i. e.* below 6·5) tend to increase the diameter, flatten and render less rigid and de-prickle the same cells.

The exceptional case of carbonic acid, which, though a weak acid, tends to stiffen and prickle washed red cells, is of special interest, in view of the important part played by CO_2 in the physiological reactions of the red cell in connection with the aëration of the blood, and as the chief stimulant of the respiratory centre.

Oxygen, which has but little effect on the pH value of the saline solution in a standardized suspension of washed red cells, tends to act in the opposite direction to CO_2—that is, to enlarge the diameter, and flatten, or, in other words, to deprickle the cells.

The effect of de-oxygenation, on the other hand, is to prickle washed cells.

It has been shown that lipoidal substances, *e. g.* soap solutions (oleate of soda ·02%) *and* proteins, *e. g.* serum albumin in solution, deprickle prickled red cells.

The question of the relative influence of these two groups of substances, and whether any portion, or how much of the action of such proteins is due to association with lipoids, has not yet been definitely determined.

The evidence brought forward suggests, however, that in the case of both substances, the deprickling action depends on the fact that both proteins and lipoids behave as *surface-tension reducing substances*, and act as such on the red-cell coat.

They produce their effects by "sitting" as it were, at the contact face between the red-cell envelope and the watery medium in which the cell floats, and also at the contact-face between the water medium and the air and the glass slide.

The actual way in which these substances (soap or protein in solution) lower surface tension at these contact faces, and bring about the spreading and stretching of the lipo-protein pellicle which forms the outer envelope of the red cell, and so produce the change in the shape of the red cell associated with the flattened disc form, is, we may suppose, by bringing about an inversion of phase, or by the interposition of water molecules between the fat molecules in the one case, and fat molecules between the water molecules in the other.

We have also seen that the washed red cell is highly sensitive to changes in temperature in the medium in which it rests. A temperature above 65° F. tends to prickle, while a temperature of below 60° F. tends to keep the washed cell in the disc form.

Since the changes in shape which accompany the corresponding change in temperature have so far only been observed in *washed cells* after removal from the circulation, we are not at present justified in drawing any inference as to the effect of change in bodily temperature on the circulating red cell. There is, however, some reason for thinking that the red cells in winter (cold weather) are on the whole more stable in preserving the disc form than in the hotter summer season.

Further, we know that, in health, the red cells vary in size, that is in diameter, under different conditions and at different hours of the day. They are, for instance, smaller in the morning than in the evening. Violent exercise increases the diameter, with a corresponding increase in volume, and an increase in the activity of the blood is associated with an increase in the size of the red cells and *vice versâ*. (See Beaumont and Dodds, 'Recent Advances in Medicine,' p. 367.)

It is of interest, therefore, to find that these clinical observations are to a certain extent borne out by the laboratory *in vitro* experiments now recorded, and that, for instance, the rendering of the saline fluid slightly acid, pH 6, by the addition of ac. sod. phosph. will restore prickled red cells suspended in it to the discoidal shape.

The observations now recorded also throw additional light on the part played by osmosis on the shape of the red cell.

We know in a general way that a hypertonic saline solution stiffens and prickles, while a hypotonic solution softens and flattens and eventually hæmolyses washed red cells, whereas in an isotonic solution, the cells retain the normal biconcave disc shape for a considerable time.

The fact that the addition of a ·02% soap solution (oleate of sod.) or blood-serum restores cells, which have been prickled by a hypertonic solution to the disc shape, shows that no irretrievable damage has been done, either to the cell envelope or the stroma.

We have also seen that when tested by the effect on the coat of the red cell of previous washing in saline solution, the chlorides of the sodium group and the earthy bases may be arranged in two series. The first, or sodium chloride group, tends to preserve the pliability and disc shape of the cell, while the second, or barium chloride group, tends to shrink, stiffen and prickle the cell.

An isosmotic solution of sod. sulphate softens and flattens, and so *de*prickles the cell and eventually produces hæmolysis.

This difference in influence between Ba chlor. on the one hand and sod. sulphate on the other is probably associated with a difference in the hydrophyl capacity of the two substances, and to the direction in which the iso-electric point is displaced on either side. All these observations may, I hope, throw some light on the action of the inorganic constituents of the blood-serum.

Finally we have seen that contact with certain living cells, especially leucocytes and cells belonging to the

endothelial reticular system, and perhaps some other cells, enables washed red cells to preserve the normal disc shape.

When we consider the necessity of avoiding a too intimate contact, and friction, between the circulating blood-cells and the fixed cells which form the cellular lining of the narrow tubes through which the red cells are transported, this double protection afforded against injury, by a non "water wetable" coating of both circulating and fixed cells, and by the presence of surface-tension reducing substances in the fluid medium, assumes increasing importance.

In the next lecture I propose to deal with the clinical and biological application of the facts and conclusions which we have been considering to-day.

LECTURE V

ON THE CLINICAL AND BIOLOGICAL SIGNIFICANCE OF THE ABOVE OBSERVATIONS

Clinical.

The fact that the mammalian red cell undergoes changes in shape when the physical or chemical composition of the environmental medium is altered, and the fact that these changes in shape are associated with changes in the diameter, size, rigidity and physical condition of the red cell, suggests that further investigation may explain certain clinical observations concerning the blood which are at present imperfectly understood.

We can, for instance, ascertain what are the diseased states in which the red cells (when appropriately treated) depart from the normal discoid shape, and become prickled or otherwise altered in form.

We can also employ these changes in shape as a means of testing the activity, not only of normal, but also of pathological body-fluids. Knowing also (though at present only to a limited extent) the relation between change of shape and other physical and chemical changes in the constitution of the cell, we may be able to link up the two sets of facts, and so get a little nearer to fundamental knowledge about cell structure, and cell metabolism.

The Influence of the Blood-Serum.

In the previous lecture I explained that, while it is the common property of most blood-sera to restore prickled or globular red cells to the biconcave disc form, different sera vary considerably in the degree to which they produce this effect.

This variation in "deprickling capacity" must depend on some difference in either the lipoid or the protein constituents of the serum, or possibly on both, or more accurately on the surface-tension reducing substances present in the serum. "Deprickling capacity" is *not* characteristic of positive or of negative sera, as tested by the Wassermann reaction.

In many diseases due to infection, as, for instance, pneumonia, and some suppurative diseases, the deprickling capacity of the patient's blood-serum may be heightened, probably owing to an increase in the emulsifying or surface-tension reducing substances present.

The same is also true of the blood-serum in cases of nephritis with albuminous urine.

In certain cases of heart disease, with obstruction to the venous circulation and cyanosis, the once-washed red cells, or in some cases even the red cells in diluted blood (*i. e.* 1 drop of blood to 2 c.c. of N.S. solution), show a tendency to prickle, although the blood-serum from the same patient deprickles foreign red cells.

The same is true in certain cases of diabetes and in the so-called "pink disease" in children.

In these diseases the tendency to lose the discoid shape is evidently associated with some abnormality in the red cells themselves, in the envelope or stroma, or both, and not in the blood-serum.

There seems to be no constant difference in deprickling capacity between the sera of persons belonging to different blood groups, that is to say, there is—as we have already seen—no constant association between *deprickling* and *agglutinative* capacity. This we should expect if the

agglutinating substance is linked up with the euglobulin constituent, and the deprickling substance with the serum albumin, or with the lipoid elements in the blood-serum.

In a previous lecture I mentioned that certain lipæmic sera are deficient in deprickling capacity.

This may depend on (*a*) the size of the fat-droplets, or (*b*) the chemical composition of the fat, or to a combination of both factors.

Lipæmic sera which, under the microscope, show extremely small fat-droplets, exhibiting Brownian movement, are not easily cleared, even by rapid centrifugalization; but if the serum is pipetted off the blood-clot and allowed to stand for some hours, then the fat-droplets, if sufficiently large, will rise to the surface and form a fatty layer, like cream on milk.

The following case is of interest in this connection:

I. D—, a male infant, aged 11 months, very anæmic, with enlarged liver and spleen, thought to be a case of Von Jaksch's disease.

The blood-serum gave a negative Wassermann reaction, and was very milky in appearance. It did not deprickle a suspension of either native or foreign washed prickled red cells.

After seven days' treatment with liver extract the blood-serum became clear and normal in appearance, and it then deprickled both native and foreign washed prickled red cells.

After *dis*continuing the liver extract for eight days, the blood-serum again became milky, or lipæmic in appearance, and again cleared when the treatment with liver extract was recommenced.

Incubation of the blood-serum for three or four hours in a test-tube, with a small piece of freshly washed pig's liver, cleared the serum.

These facts suggest that the fat, which was in excess in the blood-serum of this child, was also abnormal in constitution, and that the effect of the treatment with liver extract, both *in vitro*, and *in vivo*, was to remove

the fat from the serum by hydrolysis and to convert it into soap. Coincidently with this change, the capacity of the blood-serum to keep the washed red cells in the disc form was restored.

Thus the antecedent factor in this case was apparently some failure on the part of the liver lipase to hydrolyse the blood fat.

This action of liver extract on blood-serum will be again referred to when dealing with the action of avian blood-serum on mammalian red cells.

Although the above observations point to the deprickling influence of lipoidal or soap-like bodies in the blood-serum in this case, this does not mean, of course, that fats and soaps are the only constituents of the blood-serum which are capable of restoring prickled red cells to the disc shape. We know, for instance, that proteins under certain conditions have the same effect. It is, however, still uncertain to what extent, if at all, these two constituents are associated together by linkage or adsorption, or in some other way, in producing the deprickling effect.

It has been suggested (see Cole, 'Physiological Chemistry,' 6th edition, p. 46) that the insolubility of euglobulin in distilled water may be due to association with lipoids.

Exudates and Transudates.

Pleural Effusions.

The clear straw-coloured serum, which can be aspirated from the pleura in certain cases of pleurisy, which does not grow any organisms on culture, and from which the tubercle bacillus is only occasionally obtained, behaves like blood-serum in regard to its deprickling effect on pyridine prickled red cells (P.P.R.C.).

In some cases such a pleural effusion may show an even higher deprickling capacity than the blood-serum obtained from the same patient.

If the effusion is the outcome of a general infection, such as pneumonia, it may indeed show a very high deprickling effect (D ++++). Thus, in a case of pneumococcal infection at the height of the disease, with a non-purulent serous effusion on one side of the chest, the pleural fluid gave a very high (D +++++) deprickling effect on P.P.R.C. This effusion had a drop number of 63,* and after treatment with a small quantity of pyridine solution, the drop number rose to 89, with a coincident drop in deprickling capacity.

Ascitic Fluid.

The ascitic effusion, which occurs in cases of hepatic cirrhosis or other forms of circulatory and biliary obstruction, only slightly deprickles washed red cells, and on standing for one or two days this deprickling effect may be lost. If, on the other hand, the fluid is the result of an inflammatory process, then its deprickling capacity is more marked and more permanent.

Hydrocele Fluid.

One sample from an elderly man, consisting of a clear straw-coloured fluid with a drop number of 65, deprickled (D ++) pyridine prickled red cells.

Cerebro-spinal Fluid.

The composition of the subarachnoid fluid, which bathes the central nervous system and spinal cord, when removed by lumbar or cisternal puncture, is of much interest from our present point of view.

Normal cerebro-spinal fluid, *i. e.* cerebro-spinal fluid containing not more than ·02% of protein and between 710–750 mgm. of chlorides in 100 c.c. of fluid, with a trace of sugar, and only a few cells, when added in equal

* As tested with the stalagmometer.

volume to a drop of a standard suspension of prickled red cells on a slide, has *no* deprickling effect on the red cells. It tends, in fact, to increase the shrinking and hardening effect of the pyridine, and converts the cells into spheroidal, smooth surface, " bullet "-like cells.

Further, if a drop of such a normal C.S. fluid be added to a drop of a suspension of once-washed disc-shaped cells in N.S. solution, the cells become smaller, many are prickled, and the remainder become " bullet " cells.

Moreover, this prickling effect is not due to mere alkalinity of the fluid. The pH value of normal C.F. fluid varies from 7·5 to 8, and pathological C.S. fluid, with an equal or larger deviation from neutrality on the alkaline side, but with a high protein content, will rapidly deprickle the same prickled red cells.

Pathological C.S. fluid, on the other hand—that is, fluid obtained by lumbar puncture from a case of tubercular meningitis, meningococcal meningitis, pneumococcal meningitis and other inflammatory affections of the central nervous system, such as tetanus, encephalitis, etc. —rapidly deprickles the red cells in a standard suspension, even when it is quite clear and normal in appearance.

Small, prickled, spheroidal cells reassume the flat, biconcave disc shape ; they tend to form " rouleaux," and, if the blood-serum of the patient belongs to a blood group which normally agglutinates the same red cells, then these disc-shaped cells will show some degree of clumping, according to the amount of serum albumin in the fluid.

Such a deprickling, *pathological* C.S. fluid may be clear and normal to the naked eye, with only a few leucocytes, and a slightly raised pH value up to 7·5 or 8, but it will have an increased protein content. It will also froth definitely when air is bubbled through it from a pipette.

In normal C.S. fluid, on the other hand, the protein content will vary from ·01 to ·025 ; and the chlorides

from 710 to 750 mgm. per 100 c.c., and it will *not* froth—that is to say, the air bubbles which form, break up and disappear on the surface of the fluid, and do not remain or rise in the tube. There is, in fact, a comparative absence of surface-tension reducing substances in normal C.S. fluid. It is known that in pathological C.S. fluids the protein rise is mainly due to an increase in the globulin constituent. The normal proportion of 1 part of globulin to 7 parts of albumin is changed to 1 of globulin to 3 to 4 of albumin (see A. Cannon, 'Brit. Med. Journ.,' July 12th, 1931). A large number (some forty or fifty specimens) of normal and pathological C.S. fluids have now been examined for deprickling capacity, and, up to the present, while no pathological fluid with a definitely raised protein content has failed to deprickle prickled red cells under the conditions described, no normal fluid, *i. e.* no C.S. fluid, with a normal protein content, has shown this deprickling effect. In nearly all cases, on the other hand, the normal C.S. fluid induces prickling in a standard suspension of once-washed red cells.

In fact it is possible, by an examination of the effect of a given C.S. fluid on a drop of a standardized suspension of prickled red cells, to form a rapid preliminary, and fairly accurate judgment, as to whether a given sample of C.S. fluid should be classed as normal or pathological. Further, when this preliminary examination has been supplemented by the frothing test, and the further examination about to be described, an accurate opinion may be given as to the protein content, and the presence or absence of surface-tension reducing substances (protein or lipoids) in such a fluid.

Treatment of C.S. Fluid with Chloroform.

If, to a sample of normal (non-deprickling), and to a corresponding sample of pathological (deprickling) C.S. fluid, an equal volume of chloroform be added, and the two samples so treated be well shaken and then

allowed to stand for some hours, a small stratum or deposit of emulsified chloroform droplets will collect at the interface between the clear water layer above and the chloroform layer below. In the normal sample this is clear, while in the pathological sample, the deposit of emulsified or coated chloroform droplets is much larger, and may occupy the whole of the lower chloroform layer at the bottom of the tube.

A drop of the upper water layer from the pathological fluid, pipetted on to a slide, still deprickles prickled red cells, and continues to do so after evaporation to dryness and re-solution in water, while a drop of the chloroform layer, when allowed to evaporate to dryness, and to form a fat film deposit on the slide, will show *no* deprickling effect. In this respect pathological cerebro-spinal fluid, after treatment with chloroform, behaves rather differently, as regards the water and chloroform extract, to blood-serum, in which, as we have already seen, the choloroform extract, after evaporation, is still capable of deprickling red cells.

The deprickling action of the water extract, after treatment of the C.S. fluid with chloroform, is not due to the presence of retained chloroform in the water, because the same result can be obtained from the same fluid after evaporation to dryness and expulsion of any dissolved chloroform; nor is it owing to any accidental acidity due to the presence of HCl as an impurity in the chloroform, for the deprickling effect can be obtained after neutralization of the fluid to pH 7 or 7·5. This means that the positive result is due to the presence of surface-tension reducing substances probably (proteins) in the watery C.S. fluid.

What legitimate conclusion, then, can we draw from these experiments as to the nature of the substance, or substances, which are present in pathological, but absent in normal C.S. fluids, and which produce this deprickling effect on prickled red cells ?

From evidence already given there is reason to think

that these bodies must be *surface-tension reducing substances*, which means, in the case of C.S. fluid, that they are either *proteins*, or *fatty, soap-like* substances. The ease with which frothing can be produced in these fluids supports this suggestion. We know that the protein content is increased in these pathological fluids, but we also know that the C.S. fluid bathes the cerebral and spinal tissues, which are very rich in lipoidal contents.

The fact that previous boiling of the pathological fluid abolishes its deprickling capacity is important in this connection. Boiling, of course, coagulates and otherwise disintegrates the proteins, but boiling may also bring about the adsorption on to the coagulated protein particles, and the consequent removal (as far as deprickling effect is concerned) of any lipoidal or soap-like bodies present in the fluid.

The fact, however, that the deprickling substance is found in the water extract and *not* in the evaporated chloroform deposit supports the suggestion that the active substance, in the case of the C.S. fluid, is the protein. It seems highly probable, therefore, that the deprickling effect of a pathological C.S. fluid is mainly due to the raised protein content, although the presence of other surface-tension reducing substances, such as fat or soaps, may not be wholly excluded.

In the previous lecture I dealt with the physical condition in these three layers of liquid when different *blood-sera* of different emulsifying capacity are treated with chloroform. In the case of C.S. fluid treated in the same way, the degree to which the chloroform globules in the bottom layer are coated and stabilized in emulsion form depends on the protein content of the C.S. fluid.

Removal of Deprickling Substances by Previous Treatment with Washed Red Cells.

This conclusion, namely, that the deprickling effect of a pathological C.S. fluid is due to a raised protein

content, is confirmed by the effect produced by treating such a pathological C.S. fluid with washed red cells.

A sample of C.S. fluid with a protein content of ·15 was treated (to one quarter of its volume) with a sedimented suspension in N.S. of washed (C.B.) red cells, and allowed to stand at room temperature for twenty-four hours. A drop of the clear supernatant C.S. fluid which, *before* treatment deprickled (++) P.P.R.C. and slightly prickled W.R.C., *after* treatment failed to deprickle P.P.R.C. and prickled ++ the same W.R.C.

This experiment suggests that the protein colloidal aggregates are adsorbed to, or deposited on, the envelopes of the previously washed added red cells, and are thus removed from suspension in the fluid, which is converted, as regards protein content, into a normal C.S. fluid.

It is further interesting to find that this clearing of a C.S. fluid, as regards protein colloids, can be accomplished in the same way by treatment with washed birds' red corpuscles—that is to say, the interaction between the fluid and the red cells is not a specific one ; any kind of washed red cell, or other receptive surface, will bring about the exhaustion of the deprickling capacity.

This result differs from that obtained by treating an *agglutinating blood-serum* with washed red cells. In this case it is essential to use red cells which possess the specific agglutinogen corresponding to the specific agglutinin present in the blood-serum, if the agglutinating capacity of the serum is to be removed.

Treatment of normal C.S. fluid in the same way by washed red cells has no such effect. The absence of deprickling capacity remains after treatment, while the actual prickling effect on W.R.C. may even be accentuated.

The results obtained by treating small volumes of blood-serum and soap (ol. of soda) solutions with washed red cells also confirm the suggestion that the deprickling effect exercised by these solutions is due to the deposit

of either protein, or lipoidal aggregates (or both), on the contact surfaces of the washed red-cell envelopes.

It is, however, more difficult to bring about complete exhaustion of deprickling colloidal aggregates in blood-serum than in C.S. fluid, owing to the much higher content of these bodies in the blood-serum. In lipæmic blood-serum it seems possible to bring about exhaustion of deprickling capacity more easily. This can also be done in the case of soap solution, but a difficulty occurs here owing to the fact that a soap solution in N.S. much above ·02% rapidly hæmolyses the red cells.

Some general conclusions of importance emerge from a consideration of these results :

Firstly, that deprickling capacity, as shown by the restoration of prickled red cells to the disc form, is associated with the deposition of colloidal aggregates, protein or lipoid, on the surface of the prickled red cells ; while capacity to prickle washed, disc-shaped cells is associated with the removal of corresponding colloidal aggregates, or by alteration in their physical or chemical state.

These colloidal aggregates vary in physical configuration and size. Thus the lipoidal particles or aggregates, which are deposited from a soap solution on to the red-cell envelope, are smaller than the protein aggregates so deposited from blood-serum. Such a preliminary deposit of soap does not prevent the specific agglutination reaction when an appropriate blood-serum is subsequently added. In hæmo-agglutination of the specific kind, there seems to be not only a surface deposit of colloidal aggregates on the red cells, but also a more or less complete chemical or physical combination between the agglutinogen and the agglutinin substances.

In true or blood-group agglutination the process is not reversible. Agitation of the clumped cells only serves to increase the firmness and density of the clumping.

Secondly, the results obtained by the fractional separation of the euglobulin and serum albumen elements

from blood-serum, previously described in an earlier lecture, confirm the suggestion, reached by other observers, that true agglutination is the outcome of a union or chemical combination between protein rather than lipoidal aggregates in the red cells. This has been shown to be so by Schneider in the case of the agglutinating effect of bean extract on washed red cells.

In this connection it is interesting to find, as previously stated, that the addition of soap solution to washed red cells, with the deposition of the smaller lipoidal aggregates on the red-cell envelopes, does not in any way prevent further access to the corpuscle of the agglutinin aggregates from an agglutinating blood-serum.

Agglutination seems to take place almost equally rapidly and completely in a suspension of disc-shaped as of prickled cells, and also between individual cells of both shapes.

The Relation Between Deprickling and Agglutination.

Thirdly, the relationship between prickling or deprickling and agglutination is seen in the following experiment :

A drop of a suspension of red cells in N.S. containing a very small percentage of native blood-serum (obtained by diluting a drop of blood with N.S.) is placed on a grease-free slide. A smaller drop of the same native blood-serum previously concentrated (by evaporation of one-third of its volume of water) is placed alongside, and the two drops are allowed to coalesce under the pressure of a cover-glass.

In this way the gradual flow of the concentrated serum through the suspension of red cells will show a graded effect, from rouleaux formation to agglutination, according to the degree of concentration of the serum in the fluid in the area under observation.

In this case the agglutination effect, which should not normally take place between red cells and native serum, has been brought about by concentration of the serum

either by partial drying, or by exposure to the air, or by both means.

This experiment can be made even more demonstrative by evaporating a drop of normal blood-serum to complete dryness on a slide and then placing on it, *under* a cover-glass, a drop of the standard suspension of native washed red cells in N.S. The agglutinative effect of the dried and redissolved blood-serum will be most marked in the centre of the fluid, while rouleaux will form where the serum is less concentrated.

In this case the serum has been rendered agglutinative to *native* red cells.

The same result is obtained if dried serum is tested with the red cells from an individual belonging to a blood group in which the cells are not normally agglutinated by that serum. The concentrated serum now possesses in fact the agglutinative capacity belonging to the serum from another blood group.

The fact that a serum, so treated, retains its agglutinative capacity when restored to its original volume by the addition of water, shows that it has undergone some change other than mere reduction in volume by evaporation.

The question arises, what is the nature of this change ?

If a drop of blood-serum on a slide be inverted over a stream of ozone (for ten minutes to half an hour), it undergoes a change in consistency. It becomes more gel-like, less easily miscible with water, and when examined under the microscope shows a stringy or wavy appearance. Its pH value is reduced from, say, 7·5 to 6. Coincidently with these changes in physical or chemical constitution, serum, so ozonized, prickles disc-shaped red cells, and at the same time exercises a marked agglutinative effect on native red cells.

Serum which has been repeatedly agitated in air in a closed tube also becomes agglutinative, though to a less degree, though serum which has been submitted to reduced atmospheric pressure by pump action until the

formation of bubbles has ceased does not show such change.

Serum through which coal-gas has been bubbled does not lose its deprickling capacity, nor does it become agglutinative.

Radiation.

I have previously mentioned that blood-serum which has been radiated for half an hour by the ultra-violet light from a mercury vapour lamp in a quartz tube becomes agglutinative.

These observations suggest that in addition to concentration of volume, some change takes place in serum so treated, as the result of intimate contact with atmospheric oxygen.

The evidence does not allow at present of any definite conclusions as to the nature of this change.

If it is mainly physical in character, consisting in the formation of larger colloidal aggregates, this might possibly be associated with the increase in agglutinative capacity.

It is now known that the characters which distinguish the serum and red cells in the different blood-groups are not entirely sharply defined qualities, but that grading occurs, and intermediate sub-groups also exist. Evidence also points to the fact that the agglutinative capacity of the blood-serum of a given individual may vary from time to time. I have shown that this is true in cases of nephritis, pneumonia and other diseases (see papers on "Auto-Hæmagglutination," 'Brit. Med. Journ.,' December 18th and 25th, 1920).

The foregoing observations suggest that when a blood-serum is concentrated, or radiated in contact with atmospheric air, or when it is ozonized or oxygenated, the increase in agglutinative capacity so obtained is due to the effect of oxygen. We do not know in what way previous disease affects the agglutinative capacity of the blood-serum, or whether cyto- or bacterial toxins exercise an effect in this direction. In a case of severe puerperal

sepsis the blood-serum of the patient agglutinated the red cells of a universal donor (Group IV), and also those of another donor belonging to the patient's own group (II). This led to some doubt as to the compatibility of the blood from these donors, hence the plasma only was injected after sedimentation of the red cells, with good results. Some weeks after complete recovery this woman's blood-serum still agglutinated not only her own washed red cells, but the red cells also of the donor belonging to her own group. This condition of auto-agglutination was also present for three months after recovery in the case of pneumonia recorded in the 'British Medical Journal' paper, 1920.

The Shape of the Red Cells in Diabetes.

A number of cases of diabetes have been examined, ranging from a moderate to a highly raised blood-sugar content, associated with a clinical condition bordering on coma.

In nearly all cases the stability of the red cells in regard to shape is altered in the direction of an increased tendency to prickling.

If a drop of blood be drawn from a *healthy individual* into 2 c.c. of N.S., the cells from a drop of such a standardized suspension containing a certain amount of blood-serum, when examined on a slide—at a room temperature below 60°—show the normal disc shape.

If the suspension be centrifugalized and the supernatant N.S. fluid be replaced by fresh N.S. solution, then the cells in this standard suspension of once-washed red cells will also be disc-shaped.

But if a drop of blood from a *diabetic patient* be treated in the same way, then the cells in the standard suspension of the dilute blood and the once-washed cells will show a marked tendency to become prickled or even spheroidal in shape, when examined on the slide, under comparable conditions as to temperature, etc.

Further, if a suspension drop of such prickled cells be treated with a drop of soap solution ·02%, which deprickles normal red cells, the cells will be only deprickled with difficulty, and will require a larger quantity of soap solution than normal cells. Treatment with native blood-serum also fails to deprickle the cells, or only does so with difficulty, whereas a drop of the blood-serum from the same diabetic patient will rapidly deprickle *normal* prickled cells. This latter fact is important, because it shows that the abnormal tendency of the red cells in diabetes to become prickled, and to remain prickled, even on the addition of both native and foreign blood-serum, is not due to any deficiency of deprickling capacity in the diabetic blood-serum, but to some intrinsic abnormality or defect in the lipo-protein envelope (or stroma) of the red cells themselves. The agglutinative capacity of the red cells in diabetes to clump in the presence of an agglutinating serum, on the other hand, does not seem to be abnormal.

This instability of the red cells in diabetes, and this tendency on the part of the cells to shrink and become prickled or rounded, may be connected with a tendency on the part of the same cells to become less efficient oxygen carriers, and this again with the clinical tendency to hyperpnœa and coma in diabetes. As we shall see later, the red cells show a like change in shape in obstructive heart disease and other forms of anoxæmia. It would seem that constant exposure to the presence of reducing substances, like glucose, in the blood-serum, may lead to changes of a more or less permanent character in the structure or functional activity of the red cells.

Pernicious Anæmia.

In pernicious anæmia of the macrocytic type, the washed red cells, unlike diabetes, show but little tendency to assume the prickled or globular shape.

The cells in this disease require repeated washings in

N.S. solution and more agitation with foreign surfaces, and a larger amount of pyridine to bring about the change.

The washed red cells in cases of pernicious anæmia appear to be softer and more flabby than normal, and to take on the rigid, contracted, prickled shape with difficulty. This is in harmony with the known fact that in pernicious anæmia the diameter (*i. e.* size) of the red cells is increased (*i. e.* 8 microns).

Acholuric Jaundice.

An examination of the red cells in two cases of this disease, in the dilute blood suspension, and after once washing in normal saline, shows a marked tendency on the part of the cells to prickle.

The cells so prickled, or prickled by pyridine solution, return to the disc form on treatment with native, or foreign normal blood-serum, and soap solution, but although disc-shaped, they still remain small, the diameter of most of the cells being below the normal. There seems in fact to be some association between microcytosis and tendency to prickle, and macrocytosis and tendency to retain the disc shape, as in pernicious anæmia. With regard to the blood-serum in acholuric jaundice, samples treated with an equal volume of chloroform separate into the usual layers, but the bottom, or chloroform layer, is milky in appearance, due to the pellicles of emulsifying substance which coat the chloroform droplets. I have already referred to this point.* In normal blood-serum this bottom chloroform layer is almost clear.

It has been suggested (see Dr. Witts, 'Brit. Med. Journ.,' November 14th, 1931) that the anæmia in acholuric jaundice, and in other diseases in which the diameter of the red cells is smaller than normal, is

* It will be a matter of interest to know whether this high degree of emulsifying capacity is present in the blood-serum in all cases of acholuric jaundice.

associated with imperfect hæmoglobin formation, while in pernicious anæmia, in which the cells are macrocytic, it is the stroma which is at fault. However this may be, the evidence suggests that in the *microcytic anæmias* the red cells, originally small, tend to contract still further, and easily prickle when suspended in normal saline fluid, though they can be restored to their original size and shape by suitable treatment. If one were asked to suggest what is the underlying cause, one might perhaps regard the stroma of the corpuscles as more tense, more contractile in the microcytic than in the macrocytic condition. The familial incidence of acholuric jaundice also suggests that, along with the altered relationship between corpuscles and blood-plasma, a genetic element is present in the marrow—the factory where the red cells are formed.

There is, I think, little doubt that our conception of the function and activities of the red blood-cell has been too static. The red cell is very sensitive to changes in the medium in which it circulates. It responds to those changes by changes in size, shape, constitution, electrical charge, sedimentation rate and in other ways. The influence of the serum globulin, fibrinogen and albumin relationship on the electric charge, and the importance of the liver in connection with the serum proteins, has been alluded to by Dr. W. W. Payne ('Lancet,' January 9th, 1932).

I think we may legitimately speak of a cycle in the life of the red blood-cell corresponding to the cycle in the life-history of the leucocyte ('The Leucocyte in Health and Disease,' Lewis & Co., London). In its ordinary medium and while circulating in the blood-stream the healthy red cell is a biconcave disc ; when removed from the blood-plasma to a saline medium it tends to prickle and eventually round up and become a spheroidal cell, but it can be restored to the flat disc shape by a return to its normal medium.

In like manner, the white blood-cell, while circulating

in the blood-stream, is a rather flattened, roundish cell. Under abnormal conditions, as when emigrating from the blood-vessels during life, it becomes prickled or irregular in outline, and throws out processes or dendrites, but returns again to the rounded shape when restored to the blood-stream and body temperature.

These facts are of interest when we consider that both red and white cells (in the adult) have a common origin in the marrow.

The Effect on the Red Cells of an Increase in the Cholesterol Content of the Blood-Serum.

There does not appear to be any marked association between a rise in the cholesterol content of the blood-serum and a rise or fall in deprickling capacity. Thus, in a case of nephrosis (I. S—), with a cholesterol content of 380 mgm. per 100 c.c., the blood-serum strongly deprickled (foreign) prickled red cells, while the blood-serum in another case (H—), with a cholesterol content of 320 mgrm., failed to do so.

In a third case (A—), also of nephritis with a very high cholesterol content of over 500 mgrm., the blood-serum had no deprickling effect. While in a fourth case, also of nephritis, with a cholesterol blood content of 590 mgrm. per 100 c.c. and a blood urea content of 66 mgrm., the fresh blood-serum showed a D ++ deprickling effect.

The Effect of Cholesterol and Lecithin on Blood-Serum In Vitro.

If a small quantity of cholesterol is added to a sample of deprickling blood-serum, and the serum be then tested with washed prickled red cells, no inhibiting effect is observed on the deprickling capacity of the cholesterol serum. In fact, the deprickling effect seems to be slightly increased.

The effect of lecithin added to another sample of the

same serum is just the opposite : the cells, instead of flattening out in the disc form, become spheroidal in shape and reduced in diameter, and soon hæmolyse.

From *in vitro* experiment it would appear, therefore, that the cholesterol content in the blood-serum tends to keep the corpuscles flaccid and disc-shaped, while the lecithin content tends to stiffen and round up the cells. Further clinical and experimental investigation is, however, needed in regard to this point.

While it seems clear that the hæmolysis of red cells when immersed in hypotonic saline is not simply a question of osmosis, *i. e.* of water interchange through a semipermeable membrane, the conditions which control the escape of the hæmoglobin are not fully known.

G. S. Aslow (see ' Journ. of Physiology,' March, 1932, p. 262) does not confirm Brinkman and Van Dam's conclusion that alteration in the cholesterin-lecithin ratio in the coats of the washed red cells is the deciding factor.

The Effect of Radiation with U.V. Light on the Shape of Red Cells.

If a standard suspension of washed red cells of equal volume, one (*a*) in a *glass*, and the other (*b*) in a *quartz* tube, be equally exposed to the U.V. rays from a mercury vapour (Hanovia) lamp at a distance of 9 in. for two hours, the cells will fall in the quartz tube and form a purple-coloured sediment of deoxidized cells, while those in the glass tube will deposit as bright red oxidized cells. If, further, a drop of the cell suspension from the quartz tube be examined on a slide, the great majority of the cells will be prickled or globular, while some will be hæmolysed or ghost cells. Those from the glass tube, on the other hand, will be disc-shaped and more normal in appearance.

The disintegrative change initiated by the radiation in the quartz tube goes on after the radiation has been

cut off, until all the cells become laked. Coincidently also the N.S. solution in the quartz tube froths more when aërated than that in the glass tube.

Cells which have not been hæmolysed or irretrievably damaged can be restored to the disc shape by the addition of a little ac. sod. phos. solution in N.S., or by soap (ol. sod. sol. in N.S. ·02%) or by blood-serum.

Effect of Solar Radiation of the Body on the Shape of the Red Cells.

On May 25th, 1931, a control sample of a standard suspension of washed red cells drawn from the finger was examined at 11 a.m. The cells were disc-shaped.

After six hours' exposure of the bare arms, face and neck to bright sunshine and sky-shine, a corresponding sample of washed cells was examined from the same finger, and a majority of the cells on settling down on the glass slide became prickled.

This experiment suggests that, after the exposure of a considerable extent of skin area to sunlight, the red cells, although they remain disc-shaped while circulating in the blood, yet tend to assume the prickled shape more quickly than non-irradiated cells when withdrawn from the circulation, and suspended in N.S. solution. It is probable that the red cells from an animal, exposed to radiation for a sufficient length of time to produce extreme results on the blood, would show the prickled shape more markedly still.

Summary of Clinical Aspect.

Before passing on to consider the *biological* aspect of the problem, we may summarize the *clinical* findings.

We have seen that, while blood-sera differ in deprickling capacity, this difference is not associated with changes in agglutinative capacity, nor does it depend on the particular blood-group to which a given serum belongs. Neither does it depend on complement-deviating

properties. It occurs in both negative and positive blood-sera, as determined by the Wassermann test.

Further inquiry is very desirable as to the presence of a heightened or diminished deprickling capacity in the blood-serum in different diseases. At present we can only mention a few diseases in which any constant association has been observed between a given disease, or disease complex, and a change in deprickling capacity in the upward or downward direction.

In many of the infections, as in pneumonia, the deprickling capacity of the blood-serum, as tested on a standardized suspension of prickled red cells, is increased. This seems also to be true of nephritis with albuminuria.

In certain cases of anoxæmia from heart disease, with obstructed venous circulation, the red cells show an abnormal tendency to harden or become more tense and to prickle. The same is true of the washed red cells in diabetes, and acholuric jaundice. Further, this tendency is due (in diabetes) to an intrinsic change in the red cells themselves, rather than to any change in the deprickling capacity of the blood-serum of the patient.

In pernicious anæmia, on the other hand, the once washed red cells show but little tendency to shrink or prickle. They preserve the large, flat, disc, or biscuit shape, even when treated with doses of pyridine which would rapidly prickle normal cells. Here, again, the abnormality seems to belong to the red cells and not to the blood-serum.

While *in vitro* experiments suggest a marked difference between two samples of the same blood-serum, to one of which cholesterol has been added and to the other lecithin, no marked interference with deprickling capacity has, so far, been observed in the few cases in which a patient's blood-serum has shown a considerable rise in cholesterol content. Certain lipæmic sera, however, show a diminution in, or even an absence of deprickling capacity, though this is by no means common to all such turbid sera.

Evidence has been given which suggests that, while the presence of recognizable fat-globules in the blood-serum is associated with deficient hydrolysis on the part of the liver lipase, the retention or loss of the deprickling capacity, by such lipæmic sera, may also be associated with the chemical or physical constitution of the fat-globules present in the non-hydrolysed state.

Certain exudates, *e. g.* pleural effusions, may show a higher deprickling capacity than the blood-serum of the patient from whom the exudate has been obtained.

Normal C.S.F., *i. e.* C.S. fluid with a normal protein content, has no deprickling effect on washed prickled red cells—in fact such a fluid will even prickle once-washed disc-shaped cells.

C.S. fluids, on the other hand, with a protein content above the normal, *i. e.* over ·02%, deprickle the cells in a standard suspension of washed prickled red cells.

This difference may be used as a preliminary test by which to determine the normal or pathological character, *i. e.* the protein content, of a given sample of C.S. fluid.

Examination of the water and the chloroform extracts respectively after the treatment of such pathological C.S. fluids with chloroform, shows that (in the case of C.S. fluid) it is the protein and not the lipoid substances which are responsible for the rise in deprickling capacity. The question, however, remains whether adsorption of lipoids to the protein molecules so treated may not account in part for the change. The difference in tendency to froth on aëration confirms the suggestion that the deprickling capacity depends on the presence of surface-tension reducing substances in the fluid, probably of a protein nature, in the case of pathological C.S. fluid.

Radiation.

Finally, evidence has been given which shows that the early effect of radiation by U.V. light on washed red cells is to cause the cells to shrink or prickle and later to hæmolyse.

Exposure of a considerable area of skin surface to solar radiation, of sufficient intensity and duration, also tends to bring about a deviation in the same direction, *i. e.* away from the normal or disc shape, in the washed cells obtained from the capillaries of the skin areas exposed to such radiation.

The Biological Aspect.

We may now pass to a short consideration of certain biological aspects of the problem. By this I mean that we can make use of the facts now recorded, as to the changes in shape undergone by human and mammalian red cells, under certain known conditions, as a means of finding out whether, for instance, avian and amphibian resemble mammalian red cells in this tendency to change their shape, when their environment is altered, and, further, what effect the blood-serum of birds, frogs and fishes has on the shape of the washed mammalian red cell.

At the outset it is very necessary to bear in mind that the red cells of birds, frogs and fishes are *nucleated* cells, and that this difference in structure must be taken into account when observing the behaviour of these cells under different conditions.

If a standard suspension of washed fowl's red cells be made in N.S., as in the case of human cells, and if a drop of such a suspension be examined on a slide without a cover-glass, the cells will appear as ellipsoid flattened discs, with a smooth outline and a central nucleus. The addition of pyridine 4% solution in N.S., which rapidly prickles human red cells, has little effect in changing the outline, except that in a few cells a very fine notching of the edges can be made out when the cells are examined in a favourable position. Repeated washing in normal saline does not prickle the cells. The same is true of amphibian and fishes' red cells, though the cells of the toad and the fish (trout) show a slightly

greater tendency to prickle, *i. e.* to assume the notched shape, when treated with pyridine than those of the bird (fowl).

Along with this difference in behaviour between avian and mammalian washed red cells, as regards change of shape, we also find a striking difference between avian and mammalian (human) blood-serum, as regards de-prickling capacity.

Avian Blood-Serum.

If a drop of a clear sample of fowl's blood-serum (from clotted blood) be added to a drop of a standard suspension of prickled human red cells, no deprickling of the cells or restoration to the *disc* form takes place. Further, if a drop of the same serum be added to once-washed disc-shaped human red cells, the cells shrink and become prickled or globular in shape.

This result suggests a marked difference between avian and mammalian blood-serum as regards the presence of surface-tension reducing substances, capable of acting on the coat or the stroma of the washed human red cell. The question arises whether this difference lies in the *lipoid* or the *protein* content of the avian serum, or in both constituents. The following observation throws some light on this point :

A known volume of fowl's blood-serum is well shaken up with, and allowed to stand for some hours in contact with, an equal volume of chloroform. The chloroform may then be pipetted off, and the process repeated with a second volume of chloroform.

A drop or two of this chloroform extract, evaporated to dryness on a slide, leaves a fat film which, unlike human blood-serum so treated, has little or no deprickling effect on human red cells.

If, however, a drop of the top layer, or water extract, be evaporated to dryness, in the same way, and redissolved in water, the serum so treated, after extraction of the fat

by the chloroform, will be found to actively deprickle human red cells. Moreover, this restoration to the disc shape occurs after any acidity present in the chloroform has been neutralized, and the pH value of the serum brought to pH 7 or pH 7·5.

This experiment suggests that the absence of deprickling capacity in the fowl's blood-serum, in the fresh state, is due to the presence of a lipoid, chloroform-soluble, element which alone, or in association with the protein constituents, inhibits the normal action of the surface-tension reducing substances in the serum. The fact that the protein elements in the serum deprickle human red cells, after extraction of the fat, supports this view.

Further, if a known volume of fowl's blood-serum be treated by the Hartley process, and the euglobulin fraction be precipitated and removed, and a known volume of the filtrate containing the serum albumin be then treated with an equal volume of chloroform, in the same way as that described in the case of the fresh serum, then the water extract fraction containing the serum albumin freed from fat, after it has been neutralized, and made isotonic, will give definite indications of deprickling capacity when tested with washed prickled human red cells, though less markedly than the water extract of the chloroform-treated fresh serum.

Thus the fat deposit from the chloroform extract fails to deprickle, and the euglobulin fraction, after washing in distilled water, gives no indication of deprickling capacity.

These observations, taken together, suggest the conclusion that it is the lipoid constituent in the fowl's blood-serum which, either alone or in combination with the serum albumin, is responsible for the absence of any deprickling effect when avian blood-serum is tested with washed prickled human red cells.

This view is supported by a further accidental observation—that a sample of very turbid lipæmic blood-serum

from one particular hen, which had ceased laying, and in which the liver-cells were loaded with fat, behaved in a different way to normal avian serum. It not only failed to prickle disc-shaped human washed red cells, but it showed a slight tendency to *deprickle* washed pyridine prickled human cells. A second sample of lipæmic serum from another hen, also with a fatty liver, markedly deprickled human red cells. Although normal fowl's blood-serum fails to deprickle human red cells, it does not prevent the action of any other deprickling agent, such as human blood-serum or oleate of soda solution, if such agent be also added with the avian serum to the red cells in suspension.

Although the description I have just given of the action of avian blood-serum on human red cells is accurate for all samples of fowl's blood serum, so far tested in the fresh state, I have found that in the case of the blood-serum of certain individual fowls, both the blood-serum after treatment with chloroform, and the liquor containing the serum albumen after precipitation and removal of the euglobulin by the Panum-Hartley process, still have no deprickling capacity when tested with prickled human red cells. I am not able at present to explain these apparent exceptions to the general rule, but I am inclined to think that it is associated in some way with the functional activity of the liver in individual fowls. Another exception occurred in the case of a chicken five weeks old, ill from some intestinal infection with profound anæmia. The fresh blood-serum from this bird deprickled washed prickled human red cells.

These observations on lipæmic avian blood-serum, in relation to deprickling capacity on human red cells, are of interest when considered in connection with the absence of deprickling capacity in certain human lipæmic sera, in which, as we have seen, the nature of the lipoid aggregates plays an important part.

It is unfortunate that the absence of prickling in the case of fowls' washed red cells makes it impossible to test

the deprickling capacity of avian blood-serum on avian red cells.

This association between arrest of ovarian activity and fatty changes in the liver in the fowl, may be compared with the pigmentary change or deposit of melanin in the liver of the female toad when prevented from entering the water for the purpose of egg-laying or spawning.

Fowl's Blood-Serum and Hæmagglutination.

With regard to the agglutinin factor, fresh fowl's blood-serum and (? avian serum in general) agglutinates human (C.B.) washed red cells.

After separation of the euglobulin fraction (in the sediment) and the serum albumin fraction (in the filtrate or liquor) by the Panum-Hartley process, and after re-solution of the sediment in water, neutralization, and restoration of the isosmotic character of the redissolved fluid in both liquor and sediment, we find that, while the agglutinin is present in full strength in the redissolved euglobulin, it is absent from the serum albumin, but the deprickling element is present in the serum albumin, and absent in the euglobulin fraction.

This shows that the protein constituents in avian blood-serum behave somewhat in the same way as human (mammalian) blood-serum as far as hæmagglutination is concerned, though differently in regard to deprickling capacity.

Summary of Conclusions in Regard to the Biological Aspect.

We find, as we might indeed suspect, that not only do the red cells of birds', frogs' and fishes' blood show no tendency to prickle after washing and suspension in N.S. solution, or in the case of frogs and fishes, only a very fine crenation of the rim of the flat cell, but also that the blood-serum of the same animals exerts, in the fresh

state, and under ordinary conditions, little or no deprickling effect on washed and prickled human and other (mammalian) red cells.

Evidence has also been brought forward which suggests that this absence of deprickling capacity, at any rate in the case of fowl's blood-serum, is in some way associated with the lipoid element, rather than with the serum albumin constituent of the fowl's serum, and further that it is also associated with the activity, ? lipase content, of the fowl's liver.

In general it seems that, while it is the lipæmic human blood-serum which fails to deprickle human red cells, *i. e.* a serum in which the fat particles have not been hydrolysed into soluble soaps, in the case of avian blood-serum it is the clear serum which fails to deprickle, and the lipæmic serum which deprickles human red cells.

Specificity of the Deprickling Reaction.

Further than this we cannot at present go. The question of the specificity of the deprickling reaction as a whole still remains. That is to say, is the deprickling effect which is exercised by human and mammalian blood-serum, in restoring prickled or spheroidal human red cells to the normal disc shape, limited to the red cells of certain individuals, or to individuals belonging to certain groups, just as the specific hæmagglutination reaction is limited to individuals belonging to certain blood-groups, or is the deprickling effect a general reaction common to all red cells, and to all blood-sera ? The evidence, I think, clearly shows that the reaction in the case of mammalian blood-serum is *not* a specific one, and that the differences in deprickling capacity which we have observed in different blood-sera must be explained by variations in the kind or the amount of the surface-tension reducing substances present in the serum, either in the lipoid, or the protein constituent, or in both, rather than by the presence of any specific substance

which only acts on the red cells of certain individuals, or of individuals belonging to certain blood, or other groups, as in the hæmagglutination reaction.

But while this is true of human, and probably of most mammalian blood-sera and red cells, evidence has been brought forward which suggests that, as we pass lower down the evolutionary scale to genera and orders having remoter kinship with the human species, then incompatibility, and some degree of specificity of deprickling reaction, may be present.

For instance in the hedgehog, a primitive type of animal belonging to the Insectivora, the blood-serum, while it has no deprickling effect on washed and prickled human red cells, does deprickle native or hedgehog prickled red cells. The hedgehog's blood-serum seems to stand in an intermediate position between mammalian and avian blood-serum. Hedgehog's blood-serum has, in fact, a positive prickling and agglutinating effect on washed, disc-shaped, human cells. On the other hand, the washed red cells of the hedgehog are easily prickled by repeated washing in N.S. solution, and are deprickled and restored to the normal, biconcave disc shape, by human blood-serum or by weak soap solution.

When we pass, however, from mammalian blood-serum and non-nucleated red cells to the blood-serum and the nucleated red cells of birds, amphibians, and fishes, then we come to wider differences in behaviour which amount to what we may call specificity of reaction in the generic or racial sense. We have seen that the washed nucleated red cells, in these orders, do not prickle, or only prickle slightly at the cell's rim, and we have also found that the blood-serum in the same animals does not deprickle mammalian red cells, except under exceptional conditions, which seem to be associated with some disturbance in liver function.

I will conclude by expressing the belief that this problem of the change in shape undergone by red cells under certain conditions, and that of the capacity of the

blood-serum to restore such altered cells to the normal, biconcave, disc shape, in the case of human and some other mammalian red cells, would well repay further study. A detailed study of the problem in the human species and in other vertebrate, and perhaps invertebrate forms, would probably throw additional light on questions of racial and evolutionary kinship. Further, as we have seen, notably in the case of normal and pathological cerebro-spinal fluids, a detailed study of the reaction would throw further light on some obscure problems, bearing on the chemical and physical state of both lipoid and protein constituents in normal and pathological body fluids, and on the relationship between the red cells and the endothelial cells which form the coating of the capillary tubes in which the red cells circulate, including stasis and coagulation, and so eventually, perhaps, on the structure of the red cell itself.

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